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*Pressor substance in the cortex of the kidney.* DAVID I. ABRAMSON and BEN FRIEDMAN (by invitation). Medical Service of Mt. Sinai Hospital, New York City and the Cardiovascular Laboratory, Institute for Medical Research, The Jewish Hospital, Cincinnati, Ohio.

It has been known for many years that a pressor material can be extracted from the cortex of the mammalian kidney. Recently this fact has assumed added significance in light of the findings that in animals in which hypertension is produced by the application of a clamp to one renal artery, the pressor substance is present in greater quantity in the ischemic kidney than in the control unoperated kidney.

In the present work, an attempt was made to obtain further information concerning the mode of action of this substance. The extract is obtained from fresh rabbit kidneys in the following manner: The cortex is separated from the medulla, cut up into fine pieces and placed in two changes of slightly acidulated alcohol. The tissue is then dried and weighed, ground up in a mortar with physiological saline, placed in a mixer for ½ hour, and then centrifuged at a rapid speed for ten minutes. The supernatant fluid contains the pressor substance, and retains its potency only if acidified and kept very cold.

The pressor action of this substance was tested on anesthetized cats or dogs, using a carotid cannula and mercury manometer. Injecting cocaine in concentrations sufficient to negate the rise in blood pressure produced by tyramine does not affect the response elicited by the extract of the kidney cortex. The curve produced rises slowly and then forms a plateau for 15 or 20 minutes before returning to its previous level; a type of response entirely different from that obtained with epinephrine.

The pressor effect is specific for kidney cortex, not being elicited by extracts of spleen, liver or kidney medulla. In the intact animal it produces an increase in kidney volume and a decrease in limb volume. It is inactivated by heat.

Section of the spinal cord does not prevent the rise in blood pressure. In the isolated tail of the dog, perfused intermittently with blood, the extract produces vaso-constriction; its site of action therefore appearing to be peripheral rather than central.

*Functional organization of the medial geniculate bodies in the cat.* H. W. ADES (by invitation), FRED A. METTLER and ELMER CULLER. Uni-

versity of Illinois, Urbana, and University of Georgia Medical School, Augusta.

Bilateral electrolytic lesions have been made in the medial geniculate bodies of adult cats by means of the Horsley-Clarke stereotaxic instrument. Before and after operation, limens of auditory acuity were obtained for seven representative frequencies of the audible range by the conditioned response technique.

It has been found that small localized lesions produce markedly different loss in acuity at the several test frequencies. Thus, in one case, the loss at 2000 cycles was approximately 19 decibles; at the adjacent octaves, 4000 and 1000 cycles, the reduction was only about half as great, while at the remaining frequencies, the impairment became progressively smaller as the distance from the focal 2000 cycles increased, being least of all at 125 cycles. Representative slides will be shown.

Cats with lesions affecting the same areas display similar hearing losses with the maximum usually at identical frequencies. In this manner loci for several frequencies have been established as follows: 8000 cycles—dorsal quadrant; 4000—anterior; 2000—lateral; 1000—posterior; 500—medial. It thus appears that the loci follow a spiral course, beginning with the high frequencies in the dorsal quadrant, and circling downward to the ventral region where the lower frequencies are found.

Several conclusions may be drawn from the above results. (1) Different frequencies traverse the medial geniculate bodies by separate pathways. (2) The disposition of the several loci conforms to the pattern laid down in the cochlea and is essentially a projection of the organ of Corti.

*An attempt to correlate transportation and activity in the dog's colon.* HARRY F. ADLER (by invitation) and R. D. TEMPLETON. Departments of Physiology of The University of Chicago and Loyola University, School of Medicine.

For transporting material through the colon the duration of active periods and the appearance of certain types of activity are considered in this report. Our observations were made on five cecostomized dogs trained to lie quietly throughout an experiment. The single balloon technique was employed to record motility. When not inflated the balloon measured 10 cm. in length and 4 cm. at its longest diameter. The balloon, fastened to the end of a small rubber tube (3/32 inch bore), was inserted by way of the cecostomy into the colon to a distance of 5 centimeters. The tube, graduated in centimeters, was connected to a water manometer by way of a T tube. The balloon was inflated and deflated a few times to smooth its contour. Six centimeters of negative water pressure were sucked against the balloon for standard deflation. With the deflated balloon clamped out of the system the manometer pressure was raised to 8 cm. of water above atmospheric. Continuity between the balloon and manometer was then established and thus a closed system for recording was obtained.

Throughout the course of each 200 minute experiment, the graduated tube, which was free to move, was observed continuously. Each one half centimeter of movement was recorded at the time it occurred and correlated with the motility indicated at that instant. The tracings thus obtained were divided into four 50 minute periods.

The average duration of activity for the 40 experiments conducted was

found to be approximately the same in each 50 minute period. Forward movement of the balloon, however, as indicated by the tube movement was found to be greatest in the first 50 minutes and least in the last 50 minutes.

A more detailed study of the active periods revealed that the greatest transportation occurred in the first quarter and over 80 per cent of the total transportation occurred in the first half of each period.

Transportation was observed to be usually associated with the first part of type III activity.

*Measurements of water drinking in dogs.* E. F. ADOLPH. Department of Physiology, The University of Rochester, Rochester, N. Y.

Quantitative features of water ingestion were studied in dogs (13) to 18 kgm.) on constant diet, 4 being normal and 2 having fistulas of the esophagus. In the latter the body's water content was not influenced by the drinking, except when a tube was inserted to connect the interrupted segments of the esophagus.

The manner of drinking was measured in respect to amounts, velocities, and intervals. In extreme desiccation, 71,000 cc. in 24 hours and 6900 cc. in one draft were taken by a fistulous dog, 1800 cc. by a normal. Velocities up to 700 cc. per minute, and 190 gulps per minute, were observed. One fistulous dog when desiccated (by 4 per cent of the weight) habitually drank 300 to 1000 cc. per draft about once every 30 minutes; the other, 40 to 100 cc. about every 3 minutes. Occasionally 20 per cent of the time was spent in drinking.

After the water required for maintenance in 24 hour periods had been ascertained, diverse amounts were subtracted from the diet. Fistulous dogs sham-drank more and more as water privation progressed. On the basis of body weight losses, 7 per cent deficits were obtained; on the basis of water needed to restore balance, the extreme deficits equalled 10 per cent of the body weight. The fistulous dogs, on 46 days upon which the deficits were 2 or more per cent of the weight, sham-drank enough to equal the deficits every 40 minutes.

The normal dogs, at the completions of 24 privations of 2 or more per cent of the body weights, drank amounts of water slightly exceeding the deficits (by 17 per cent). These amounts were gulped continuously and in less than 5 minutes, a time within which little or no absorption from the alimentary tract could occur; thereafter water was refused for one hour or more. The fistulous dog provided with connector tube gauged the deficits with similar accuracy.

*Volume and pressure changes in terminal lymphatics.* LANE ALLEN (introduced by W. F. Hamilton). Department of Anatomy, University of Georgia School of Medicine, Augusta.

In previous studies the volume of subserous lymphatics was observed to increase during stretching of tissues, resulting in a relative negative pressure effective in "sucking" suspensions through peritoneum and into the lymphatic lumen. This report is an extension of these observations on the mechanism of lymphatic absorption.

Histological study of the dorsal peritoneum of the frog showed definite intercellular points of absorption beneath which the endothelium bulges into the lymphatic lumen suggesting a valve-like action. The demonstrated valve-like action of these membranes seems to be no essential

part of the mechanism since direct observations on the lymphatics of the rabbit's diaphragm showed that these membranes are not subject retrograde pressures during compression of lymphatics, being protected by the terminal valves. This suggests that the primary function of valves is to permit the establishment of negative pressures in lymphatic terminals.

Similar pressure and volume relationships were demonstrated in other tissues. The lymphatics of the frog web were injected by placing suspensions over a small puncture and stretching the tissue.

Negative intra-lymphatic pressures from minus 0.5 to minus 3.0 cm. of water were determined.

Increase in lymphatic volume during stretching of tissue is made possible by histologically demonstrable connective tissue attachments.

*Physiological actions of phenethylamine and phenethanolamines.* GORDON A. ALLES and PETER K. KNOEFEL. Pharmacological Laboratory, University of California Medical School, San Francisco; Laboratories of George Piness, M.D., Los Angeles; and Department of Physiology and Pharmacology, University of Louisville Medical School, Louisville, Ky.

Comparative pressor actions of phenethylamine and the dextro, levo and racemic forms of betahydroxyphenethylamine (phenethanolamine) were studied in cats, dogs and rabbits. In cats and dogs, phenethylamine is usually the most active pressor of these compounds, but in rabbits the phenethanolamines are relatively more active, the levo form being 5 to 10 times more active than the dextro form, which is comparable to phenethylamine. The direct actions of these compounds upon the cardiac mechanisms in the different animals do not account for the difference in their relative pressor activities in rabbits as compared with cats and dogs.

Analysis of the pressor effects of these compounds and their effects on isolated rabbit intestine and frog rectus abdominis muscle indicate phenethylamine chiefly exhibits "nicotine-like" actions, while the phenethanolamines chiefly exhibit "epinephrine-like" actions. A comparison of the pressor activities of nicotine and epinephrine in cats, dogs and rabbits showed that the ratio of pressor activity of nicotine relative to that of epinephrine in the rabbit is but 1/50 of the similar ratio for cats or dogs. Since the pressor action of phenethylamine is chiefly "nicotine-like," while that of the phenethanolamines is chiefly "epinephrine-like," the differences in their comparative activities in cats and dogs, as compared with rabbits, is explained.

Contrary to most past reports, the physiological actions of phenethylamine and the phenethanolamines are such that betahydroxyl substitution in phenethylamine does modify the physiological activity appreciably, leading to an increase in "epinephrine-like" activity and a decrease in "nicotine-like" activity. This is true whether the betahydroxyl substitution leads to a chemical structure exhibiting dextro or levo optical rotation, though quantitatively the "epinephrine-like" activity is greatest with levo-phenethanolamine.

*The mode of action of the cardio-inhibitory substance.* RICHARD ASHMAN and JAMES L. GOUAUX (by invitation). Department of Physiology,

Louisiana State University Medical Center, and the Heart Station, Charity Hospital of Louisiana, New Orleans.

"There are two ways in which the A. C. substance could exert its inhibitory effect" on the pacemaker. "(a) It could check the building up of excitement throughout the cycle. (b) It could antagonize the action of excitement without affecting or being affected by its production." (Brown and Eccles, J. Physiol. **82:** 233, 1933.)

Our experiments support their conclusion that the second alternative is the correct one, and extend the analysis to include inotropic and dromotropic effects.

1. *Chronotropic.* In a typical experiment single maximal break shocks were applied to the peripheral end of the cut right vagus of a urethanized dog. The uninhibited auricular cycle was 420$\sigma$. The average prolongation of the included cycle, when shocks were applied about 10$\sigma$ after the beginning of the P wave, was 145$\sigma$; when shocks were applied 200$\sigma$ after P, 300$\sigma$; and when applied 235$\sigma$ after P, the prolongation of the included cycle averaged 65$\sigma$, while the subsequent cycles were prolonged 100$\sigma$.

2. *Dromotropic.* In one experiment, make-break shocks, applied to the dog's left vagus at intervals of about 750$\sigma$, induced A-V block, while producing little effect on heart rate, the auricular cycles ranging from 470$\sigma$ to 540$\sigma$. Stimuli applied in the range 540$\sigma$ to 330$\sigma$ before the apex of P prolonged the P-R interval. In the interval from 320$\sigma$ to 170$\sigma$ before the P apex, most of the auricular impulses were blocked. Slightly later stimuli prolonged the P-R, while still later ones produced no effect. As with the chronotropic effect, we see that inhibitory substance present throughout the cycle produces less dromotropic effect than that present during a fraction of the cycle.

3. *Inotropic.* A clamp at the base of the turtle's auricle may block the contractions while the inhibitory nerve fibers yet conduct impulses (Garrey). Such a quiescent auricular appendage may be allowed to rest for many seconds, yet vagus stimulation may produce a profound inotropic effect, which sometimes weakens the first contraction of an induced series as much as the subsequent ones. The vagus "antagonizes the action of excitement" in a muscle which had fully "recovered."

*The parasympathetic-like effect of splanchnic stimulation on pancreatic secretion.* B. P. Babkin, Catherine O. Hebb (by invitation) and Maria A. Sergeyev (by invitation). Department of Physiology, McGill University, Montreal, Canada.

Rhythmical stimulation of the splanchnic nerves with an induction current applied above or below the diaphragm in the dog elicits from the pancreas a scanty secretion, which is rich in organic material and enzymes, and is thus qualitatively similar to the secretion elicited by vagal stimulation. The histological picture of the gland (in dogs and cats) after splanchnic stimulation is also similar to that seen after vagal stimulation. In experimental analysis of these phenomena the following observations were made: Physostigmine increases both the vasoconstrictor and the secretory effect of splanchnic stimulation. Atropine does not abolish the vasoconstrictor effect but it does abolish the secretory effect. Cocaine enhances the vasoconstriction while not affecting the secretory response of the gland under stimulation. Painting of the coeliac ganglion with nicotine prevents vasoconstriction entirely but appears to increase the secretory effect of the splanchnic nerves. Administration of adrenaline

does not provoke a secretion of pancreatic juice and inhibits the discharge of zymogen granules from the acinous cells. Moreover it has been found that the secretory effect of splanchnic stimulation is enhanced by removal of the adrenal gland of the corresponding side. These and other data warrant the conclusion that the nerve fibres which reach the pancreatic acinous cells *via* the splanchnics are cholinergic in type and that their postganglionic fibres presumably are represented by short neurons within the gland itself.

*The effect of a high glucose and a high fructose diet on the body weight and on the fat, glycogen and nitrogen content of the liver and body of the albino rat.* GEORGE BACHMANN, JOHN HALDI, WINFREY WYNN (by invitation) and CHARLES ENSOR (by invitation). Laboratory of Physiology, Emory University School of Medicine, Atlanta, Ga. (Read by title.)

The experiments were carried out on Wistar colony rats. Litter mates were put on separate diets at the time of weaning, differing only in that one diet contained 68 per cent of glucose and the other the same percentage of fructose. Eighteen animals were used, 8 males and 10 females. The food mixture had the same caloric value. The paired method of feeding was followed and the animals kept on the diet for 70 days; they were weighed daily. At the end of the experimental feeding they were allowed to fast for 24 hours, killed by a blow on the head, the liver quickly removed and this organ and the remainder of the body cut and placed in potassium hydroxide for analysis of fat, nitrogen and glycogen.

The results showed that the average increase in weight of the paired animals was the same on the glucose and fructose diet, although the males gained more rapidly and their final weight exceeded that of the females. When calculated per hundred grams body weight, the average glycogen and nitrogen content of the entire body was the same in the glucose and the fructose-fed animals; the amount of fat was, however, significantly greater in the glucose-fed rats.

The liver was over 20 per cent heavier in the fructose-fed animals. The percentage of fat in the liver was, however, higher in the glucose-fed animals. There was no significant difference in the percentage of the glycogen and nitrogen of the liver with the two diets.

*Effect of nicotine on the oxidation of glucose.*[1] Z. BAKER (by invitation) and H. E. HIMWICH. Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y.

Previous respiratory studies from this laboratory disclosed that brain tissue in the presence of nicotine oxidizes glucose, but not lactate. In the present investigation, quantitative determinations were made for glucose, lactic acid, and oxygen consumption in order to establish a balance. Nicotine was used in such concentration (0.03 M) as to cause practically complete inhibition of lactate oxidation. It was found that the concentration of nicotine employed in these experiments stimulated the aerobic formation of lactic acid. This lactic acid accumulated, since it was not oxidized. The decrease of glucose could be accounted for by the increase in lactic acid and the oxygen consumption. Glucose was therefore oxidized without passing through an intermediary lactic acid stage. The results with nicotinic acid are similar to those obtained with nicotine.

[1] Aided by a grant from The Child Neurology Research.

Nicotinic acid amide has little effect. These studies are being extended to other tissues, and to other substrates, fructose, lactic, pyruvic and succinic acid. Similar results were obtained with diabetic brain.

*Simultaneous registration of the pressure and volume pulses of the thoracic aorta.*[1] R. BALLIN (by invitation) and L. N. KATZ. Cardiovascular Dept., Michael Reese Hospital, and the Dept. of Physiology, University of Chicago.

In the anesthetized dog with chest open and artificial respiration, an intact segment of the descending aorta is freed from the intercostal arteries and enclosed in a specially designed tight-fitting oncometer, which does not interfere with the free flow of blood. The volume changes of this oncometer are recorded by means of a Frank segment capsule, simultaneously with the intravascular pressure in the aortic segment. The pressure is recorded by means of the Hamilton manometer, and both pulses are recorded on photographic film. The curves are calibrated and parallax is avoided.

The simultaneous volume and pressure pulses of the thoracic aorta were recorded under a variety of conditions. It was found that as the diastolic and mean aortic pressures increased, the ratio of $\frac{\text{pulse volume}}{\text{pulse pressure}}$ decreased, though in a non-linear fashion. Under many of the conditions investigated, the start, peak and dicrotic notch of the volume pulse preceded corresponding points of the pressure pulse. The factor causing the time lag between the two pulses may be the fact that the opening of the needle by which pressure was recorded was directed down-stream, or the pulse transmission time from the upper end of the oncometer to its middle where the pressure needle opened.

The P/V curve of the aorta during the heart cycle was determined, and this was found to vary under different experimental conditions. It would appear that at least two factors are concerned in determining the P/V curve, namely: 1, the volume-elasticity coefficient of the segment of aorta enclosed in the oncometer, and 2, the fluctuations in kinetic energy occurring in this segment during the heart cycle.

*Influence of vitamin deficiencies on appetite for particular foodstuffs.* BRUNO BARELARE, JR. (by invitation), L. EMMETT HOLT, JR. (by invitation) and CURT P. RICHTER. Psychobiological Laboratory, Phipps Clinic, and the Harriet Lane Home for Children, Johns Hopkins University, Baltimore, Md.

It has previously been shown (Richter, Holt and Barelare) that rats on a diet deficient in the B group of vitamins, and given a free choice of other dietary essentials, show a decreased appetite for carbohydrate, but an increased appetite for fat. When these animals are given access to yeast, large amounts are immediately consumed, the appetite for carbohydrate is promptly restored and that for fat is diminished. These observations are in harmony with current views as to the part played by vitamin B in the oxidation of carbohydrates; failure of appetite for carbohydrate appears to be related to the inability on the part of the deficient animals to utilize it. The increased appetite for fat is in agreement with the "vitamin B sparing action" of fats noted by Evans and Lepkovsky.

Since in the foregoing and in other experiments, the appetite of the rat

[1] Aided by the A. D. Nast Fund for Cardiac Research.

seemed to be a fairly accurate guide to his nutritional requirements, we extended our observations to avitaminosis A. It was thought that the dietary choices of rats during the deficiency period and later during the corrective period might provide an indication of the metabolic fault in this type of deficiency. Rats were given a choice of the following foods in separate containers: casein, sucrose, olive oil, sodium chloride, calcium lactate, disodium phosphate, wheat germ oil, dried Baker's yeast, cod liver oil, and water. On this diet they grew satisfactorily and appeared to be in perfect health. When cod liver oil was withheld, symptoms of avitaminosis A made their appearance. These rats showed a decreased appetite for fat and an increased appetite for carbohydrate. When cod liver oil was made accessible to the animals, the disappearance of the deficiency symptoms was associated with an increased appetite for fat and a decreased appetite for carbohydrate. This suggests that animals with avitaminosis A either require more carbohydrate or experience difficulty in utilizing fat.

*Hyperthyroidism and carbohydrate metabolism in the depancreatized dog.* S. B. BARKER (introduced by W. H. Chambers). Department of Physiology, Cornell University Medical College, New York.

It has been found that the dog loses its ability to oxidize administered carbohydrate in the early stages of fasting after pancreatectomy, but that prolonged inanition returns this capacity. The most striking chemical finding in this condition is an increased creatinuria, indicating possible breakdown of some fundamental body process. The administration of desiccated thyroid substance was used to induce in depancreatized dogs an increased endogenous creatinuria unaccompanied by the premortal complex. The response of three such animals to ingested glucose was studied before and after the production of hyperthyroidism. In both series of experiments there was no rise in R. Q. outside the diabetic range of 0.70 to 0.74, and recovery of extra sugar in the urine was 92 to 104 per cent. Since these are the most direct criteria for the evaluation of carbohydrate oxidation, it may be concluded that there was no burning of the administered sugar, even though the usual diabetic creatinuria was increased as much as 10 to 15 mgm. per hour in the thyroid series. This evidence indicates that the premortal restoration of carbohydrate oxidation is not directly bound up with the creatine mechanism.

In the hyperthyroid experiments only, nitrogen- and acetone-sparing effects were observed, in contrast to the lack of changes in R. Q. and recovery of administered glucose. These phenomena have been encountered previously, in nephrectomized-depancreatized and in phlorhizinized-depancreatized animals, although in these cases also there was direct evidence that all of the sugar given could be accounted for. Since the animals' heat production never fell off progressively, it is obvious that, in the absence of carbohydrate oxidation, simultaneous nitrogen- and ketone-sparing cannot signify decreases in both protein and fat catabolism. It is probable that at least the acetone-sparing is an unexplained adjustment to the sudden influx of exogenous carbohydrate.

*The utilization of β hydroxybutyric acid by the heart in the heart-lung preparation.* RICHARD H. BARNES (introduced by M. B. Visscher). Department of Physiology, University of Minnesota.

Studies of disappearance of β hydroxybutyric acid from the blood and tissues of the heart lung preparations have shown that as much as 300 mgm. per hour may disappear when its concentration is 100 mgm. per cent in the blood. The simultaneously determined total oxygen consumption in such an experiment is about 600 cc. per hour. The ketone body disappearance is equivalent to one-half of the total metabolism in such an instance. The metabolism of the lungs and the blood must be taken into account in these experiments. Control studies using pump perfusion of the lungs showed that the lung and blood metabolism is about one-fifth of the total for the heart lung preparation. The loss of ketone bodies from the blood and lungs was in about the same ratio as compared with the heart lung. It is concluded that the heart muscle metabolizes β hydroxybutyric acid in large quantity when that substance is present in considerable concentration. In preliminary experiments no relation was found between the blood glucose level and the rate of utilization of β hydroxybutyric acid.

*Effects of lesions of the posterior longitudinal bundle system in Macacus rhesus.* S. E. Barrera and A. Ferraro (by invitation). New York State Psychiatric Institute and Hospital, New York City.

The posterior longitudinal bundle fiber system contains fibers connecting the vestibular nuclear groups with the cranial nerves nuclei associated with movement of the eyes. These fibers are both crossed and uncrossed. Sections of these fiber systems of one and both sides were performed usually between the sixth and third nerve nuclei. In addition, vertical longitudinal section was made of the fibers crossing the midline to enter the opposite posterior longitudinal bundle fiber system. In lesions of these various structures, the group of symptoms was found which was considered to be "vestibular" in type. These manifestations included falling, postural asymmetries, asymmetries in movement of the body and nystagmus. The directional aspects vary with the location of the lesion. Incoordination of the eye movements was the rule and abnormalities in induced nystagmus were the usual findings. Serial section anatomical control of the lesions were made using the Marchi and Weigert techniques.

*The effects of partial gastric resection and posterior gastro-enterostomy on gastric motility in dogs.* Louis E. Barron (introduced by George M. Curtis). Department of Surgical Research, Beth Israel Hospital, Boston, and the Department of Surgery, Yale University, New Haven, Conn.

In the present investigation, studies were made to determine the effects of partial gastric resection and of posterior gastro-enterostomy, without pyloric obstruction, on the motility of the stomach in ten dogs. All observations were made by the balloon and kymograph method after a fasting period of eighteen hours. The total duration of each experiment was approximately three hours, and the results obtained on each animal were tabulated for comparative study. Partial gastric resection and posterior gastro-enterostomy respectively were performed after an adequate number of control observations. Following a postoperative convalescent period of about two weeks, studies were resumed and kymographic tracings were made at frequent intervals for about four months. The results

of the control studies revealed considerable variation, the motility ranging from low grade fluctuations in gastric tonus to contractions of low amplitude. At no time, however, was there any deviation from the normal. Following partial gastric resection, there ensued an abrupt change in the activity of the remaining portion of the stomach. This was manifested by a definite increase in the amplitude of the contractions, frequently to as high as fourteen to fifteen centimeters. Contractions of this amplitude often persisted throughout an entire observation period of three hours. Occasionally the motility was so intense that recording on the kymograph was impossible. This marked increase in the amplitude of the contractions were observed as late as four months after partial gastric resection. Occasionally the motility was low grade and resembled that noted during the control period. This, however, did not appear with sufficient frequency to obscure the true results of partial gastric resection.

The activity of the stomach after posterior gastro-enterostomy was definitely unlike that noted above. The motility was low grade, and frequently discrete contractions were absent during an entire experiment. Low grade fluctuations in gastric tonus, interspersed with contractions of low amplitude, were often observed. On the whole, the gastric motility subsequent to posterior gastro-enterostomy did not vary appreciably from that observed prior to this operation. In many instances it was even below normal.

*Determination of the oxygen dissociation curve of oxyhemoglobin by a new method.*[1] J. PERCY BAUMBERGER. Physiology Department, Stanford University, Calif.

The absorption of monochromatic light, of a suitable wavelength, by a hemoglobin solution or blood cell suspension is measured photometrically during the course of removal of oxygen from the solution by the respiration of yeast cells and at the same time the oxygen tension in the solution is determined by means of a dropping mercury electrode.

The dropping mercury electrode is a cathode on which the oxygen diffusing to the surface of each drop is electrolytically reduced. The accompanying current actuates a galvanometer, giving deflections proportional to the oxygen tension.

Living yeast cells are added in such concentration as to bring about the complete reduction of the oxyhemoglobin solution in a convenient length of time. The pH is buffered at a suitable value. Glucose may be added to speed up the respiration of the yeast so that the number of these cells necessary may be kept at a minimum.

The excursions of the light beams, of the galvanometers in the photometric and dropping mercury electrode circuits, are photographed on a moving film, so that a record is obtained which shows light absorption and oxygen tension against time. Corresponding points are then measured from the record and by applying Beer-Lambert's Law the per cent saturation of hemoglobin with oxygen is calculated for any oxygen tension.

The method makes it possible to obtain the oxygen dissociation curve of oxyhemoglobin in a few minutes without using any gas mixtures and requires only a fraction of a ml. of blood.

[1] This work was supported by a grant from the Rockefeller Foundation.

*Cardiovascular changes in a hot room.* H. C. BAZETT, J. C. SCOTT and F. W. SUNDERMAN (by invitation). Departments of Physiology, Medical School, University of Pennsylvania and Hahnemann Medical College.

Two subjects were maintained in a hot air conditioned room at 32.5° during the day and at 31° at night with 36 per cent humidity. One subject remained for five days and after thirty hours cooling for a second period of four days, the other for the first five day period only. The blood volume increased in each subject by about one liter, and the weight showed a corresponding rise; these data are reported separately (see F. W. Sunderman et al.). The changes in blood volume were accompanied by considerable cardiovascular adjustments. Observations were made on pulse rate, blood pressure and pulse wave velocity; cardiac output was determined both by acetylene and by calculation from the pulse pressure. The water intake, volume of urine, water loss from the skin and body temperature were also measured.

The heat produced only minor changes in rectal temperature. Initially one subject became inefficient with a considerable increase in pulse rate both lying and standing; in both subjects the increase in pulse rate on standing became progressively less as adaptation developed, and the rate in one subject fell much below control levels. A similar slowing of the standing pulse rate has been found in the summer.

With adaptation to heat systolic pressures and pulse pressures observed during standing increased to above control levels. Stroke volume on standing was much below that observed lying down but this reduction decreased as adaptation developed. Arterio-venous oxygen differences with the subject standing at first progressively increased; at the end of the period however they appeared to be again decreasing.

Cardio-vascular behavior varies according to the external temperature and to the degree of adaptation; such differences profoundly modify reactions to tests for fitness such as those of Schneider and Crampton. One factor modifying such adjustments must be the total blood volume, but the correlations are too complex to warrant for the present even tentative hypotheses. Our thanks are due to the Faculty Research Fund of the University of Pennsylvania for some financial support and to the General Electric Company for an air-conditioning unit.

*Effects of high oxygen pressure on isolated tissue.* JOHN W. BEAN and DAVID F. BOHR (by invitation). Department of Physiology, University of Michigan.

Contractions of noncirculated frog muscle, elicited by direct, motor nerve, and reflex electrical stimulation were recorded. The test preparations were mounted in a compression chamber and subjected to oxygen at from 5 to 6 atmospheres pressure. Test and control records were taken practically simultaneously.

In experiments on direct muscle stimulation electrodes were placed on the nerve free end of the sartorius muscle. Stimulation (a block of 10 single shocks 3 sec. apart) was applied at 15 minute intervals. Test muscles, after an inconstantly appearing initial increase in height of contraction on compression, showed a pronounced decrease, to complete cessation of contraction as the pressure was maintained. The onset of this decrease occurred as early as one hour after compression—usually it was more

delayed. Decompression was frequently followed by recovery, dependent upon the length of the exposure and the individual susceptibility to high oxygen. Repeated exposures had cumulative effects. The control preparations continued active for as long as twenty-four hours.

The sartorius muscle-nerve preparation gave results essentially similar to those of the muscle preparation. If high oxygen has some deleterious effect on nerve or neuro-myal junction, apparently it was masked by the action on the muscle.

For reflex studies the semitendinous muscle, spinal column with cord, afferent and efferent nerves were isolated and the whole subjected to high oxygen. While conclusive evidence regarding the reflex changes are wanting at this stage of the investigation the results strongly suggest that high oxygen adversely influences the noncirculated reflex arcs more rapidly than the other preparations, prolongs the reflex latent period and diminishes the after discharge of the arc.

The experiments on the muscle and muscle nerve preparations indicate that in addition to disturbing the transport of carbon-dioxide in circulated tissues as was concluded from earlier experiments, high oxygen exerts direct effects on the tissues themselves, conceivably through some influence on enzymatic processes involving glutathione or even through cellular damage.

*The digestibility of raw potato starch.* J. M. Beazell and C. R. Schmidt (introduced by L. A. Crandall). Department of Physiology, Northwestern University Medical School, Chicago.

It appears to be widely accepted at the present time that large quantities of raw starch can be digested and utilized by man. To test the accuracy of this impression the digestibility of raw potato starch was determined both in vivo and in vitro.

In vitro experiments: The quantity of reducing sugar produced by incubating raw potato starch with an excess of pancreatin for various periods of time was compared with that obtained by similar treatment of cooked potato starch. Considering the values obtained with the cooked starch to represent total possible digestion, it was found that approximately 6 per cent of the raw starch was digested in three and one-half hours, and that increasing the incubation period up to 20 hours did not significantly modify this value.

In vivo experiments: Ten normal medical students were given a diet containing 170 grams of raw potato starch per day for a three day period. It was found that an average of 40.3 per cent (15.4 to 57.3 per cent) of the ingested starch was lost in their feces. In a second experiment on five of the subjects large doses of a potent diastatic enzyme were given with the diet without any effect on starch utilization. However, when the diet was frozen the average quantity of starch lost in the feces by five subjects was only 26 per cent (18.5 to 39.2 per cent). All of the subjects complained of a distressing flatulence, the severity of which was roughly inversely proportional to the quantity of starch lost in the feces. The flatulence was definitely more marked when the subjects were on the "frozen diet." This suggests that freezing ruptured the fiber envelope of the starch cell, thus exposing the starch not only to the digestive enzymes, but to bacterial fermentation as well.

It is apparent that large doses of potato starch are but poorly tolerated

by normal human subjects. Furthermore, the evidence points to the fact that bacterial fermentation plays an important rôle in the digestion that does occur.

*The action of cyanide and of oxygen lack on glomerular function in the perfused frog's kidney.* L. V. Beck (by invitation) and A. N. Richards. Laboratory of Pharmacology, University of Pennsylvania, Philadelphia.

The oliguria frequently produced in the surviving frog's kidney by arterial perfusion with cyanide-Ringer or with oxygen-free Ringer is, as a rule, not accompanied by a corresponding decrease in arterial perfusion flow. This phenomenon, ascribed by some to depression of secretory processes in the glomerular membrane, has been made the basis of their belief that glomerular urine is a secretion and not a filtrate. Our experiments, like those of Adolph on asphyxia, indicate that arterial vessels which supply glomeruli are constricted by CN or $O_2$-lack whereas extrarenal vessels (e.g., lumbar arteries, ureteral branches of the urogenital arteries) simultaneously perfused, are not constricted or are dilated. In consequence, glomerular perfusion and glomerular capillary pressure may be decreased by these agencies without corresponding decrease in total arterial perfusion flow and the phenomenon referred to is not inconsistent with the doctrine of glomerular filtration.

*Time factors in thirst.* R. T. Bellows (introduced by E. F. Adolph). Department of Surgery, Neurosurgical Division, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Dehydrated dogs have the facility of drinking their water deficit quite accurately within five minutes (Adolph). Observations made on a dehydrated dog with a fistula of the esophagus indicated that sham-drinking apparently satisfied thirst temporarily. The gauge of water requirement was faulty, however, for the animal at one draft sham-drank about $2\frac{1}{2}$ times as much water as he needed. Since the rate of sham-drinking was the same as that of actual drinking, the dog sham-drank for longer periods than 5 minutes after a certain degree of dehydration. The water requirement and the rate of actual drinking were determined by inserting a connecting tube in the fistulae and allowing the dog to drink.

Administering the estimated deficit of water by esophagus satisfied thirst only after a delay of more than 10 minutes. If allowed to sham-drink during this delay period, the dog sham-drank, not the usual excessive amount of water, but the exact amount of his deficit.

These observations indicate the operation of at least two supplementary factors in satisfying the thirst and gauging the water need of dehydrated dogs. One factor may be localized in the mouth and pharynx associated with the acts of gulping and swallowing water. The other factor is operative below the level of the fistula in the upper esophagus.

Water-drinking may occur in the absence of a water deficit. A temporary intense thirst was evoked in the adipsic fistulous dog by the intravenous administration of hypertonic solutions. The rates of sham-drinking roughly paralleled the rates of diuresis and, like the diuresis, sham-drinking was self-limiting without water ingestion. Sham-drinking as well as diuresis was greater after 20 per cent sodium chloride than after 40 per cent urea. Sham-drinking was initiated 1 to 2 minutes after completion of the intravenous administration of sodium chloride, after ten

minutes following urea injection. The rapidity of the action of sodium chloride and the greater intensity of the thirst it provoked may mean that the action of sodium chloride is not entirely the result of changes in osmotic pressure.

*Relationship of polydipsia and polyuria in diabetes insipidus.* R. T. Bellows (introduced by E. F. Adolph). Department of Surgery, Neurosurgical Division, University of Rochester School of Medicine and Dentistry, Rochester, N. Y. (Demonstration.)

Diabetes insipidus was produced in dogs by cauterizing the tuber cinereum by the transbuccal route. Several observations have induced the author to believe that the polyposia (<Gr. much + drinking) is a more prominent factor than the polyuria. When the water intake was restricted to the normal amount in a diabetic dog, the polyuria vanished and the weight remained constant. When the water intake was unrestricted the animal gained weight, indicating that a gain in water content was associated with polyposia. An esophageal fistulous dog, which could be kept adipsic on an appropriate water intake, sham-drank water persistently after the tuber cinereum was cauterized, although there was no polyuria and the body weight remained constant on the same water intake.

In another experiment a loose ligature was placed around each renal artery. After diabetes insipidus had been produced the ligatures were tightened without anesthesia, producing a complete and sudden anuria. The dog continued to drink water four to five times in excess of the prediabetic intake until the body weight was appreciably increased. Thereafter the intake rapidly diminished until death ensued.

When the drinking water was replaced by an unlimited amount of sodium chloride solution the polyposia and the polyuria were enormously increased. One dog drank his weight of saline solution in 24 hours. When the saline solution was restricted to the average amount of water the dog drank in 24 hours, the rate of consumption was accelerated and there was a net loss of water. This condition persisted as long as the intake insisted of saline solution. Upon restoring the drinking water there was a retention of water, after which water was ingested at the usual rate.

*Regeneration of the third cranial nerve in monkeys.* M. B. Bender (introduced by J. F. Fulton). Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Five or 6 weeks after intracranial section of one oculomotor nerve, monkeys showed abnormal movements of the globe and ptosis of the upper eyelid. The movements were not conjugate with those of the normal eye, *i.e.*, when the monkey looked down (normal eye), the affected eyeball turned nasally and its eyelid moved upward. When the animal directed its gaze laterally (requiring action of the formerly paralyzed internal rectus muscle), the affected levator palpebrarum contracted. Eserine given parenterally exaggerated these abnormal movements. The same type of abnormal upward movement of the eyelid was obtained by electrical stimulation of the cortical eyefields. If the regenerated nerve was stimulated (i) proximal to the scar, the globe rotated nasally, the lid moved upward, and the pupil constricted slightly; (ii) if stimulated distal to the scar the same reactions occurred, but more conspicuously. Section of the

reunited nerve abolished all movements and the ophthalmoplegia reappeared. Similar observations have been made in a chimpanzee (Bender and Fulton, *J. Neurophysiol,* **1:** 1938, no. 2). Incomplete section of normal oculomotor nerve resulted in a rapid recovery without the appearance of abnormal movements. Section of ipsilateral trochlear, trigeminal, facial and cervical sympathetic nerves did not visibly alter the abnormal movements, nor were they affected by stimulation of these structures.

The order of visible recovery in function following section of this nerve was (i) disappearance of ptosis; (ii) inward movement of the globe; (iii) decrease in pupillary diameter; (iv) pupillary reactions to light, in accommodation and lid closure. Occasionally the pupil constricted slightly as part of an abnormal movement. Vertical movements were not seen. Only after the inferior rectus muscle had been cut was vertical movement seen by action of the uncut antagonist (superior rectus muscle).

It is concluded that the abnormal contractions in ocular muscles after oculomotor nerve lesions are the result of indiscriminate regeneration of axis cylinders. An impulse appropriate for the inferior rectus muscle is shunted at the scar to axis cylinders which have branched and regenerated in every direction, causing all muscles and sometimes the sphincter pupillae to contract simultaneously. Occasionally the pupil constricts slightly.

*A comparison of the estimation of basal metabolism from (a) "surface area" and (b) the formulation of Talbot, Wilson and Worcester.* JOSEPH BERKSON and WALTER M. BOOTHBY. Division of Biometry and Medical Statistics, Section on Clinical Metabolism, The Mayo Clinic, Rochester, Minnesota.

A continuation of our previous studies respecting the age changes of basal metabolism, the litter measured as calories per hour per square meter of body surface (DuBois) necessitated an examination of the formulation of Talbot, Wilson and Worcester[1] in which "surface area" is rejected in favor of an estimation from body weight. We examined analytically and statistically data from our own observations, the observations of other workers, and the data used by Talbot, Wilson and Worcester, and reached the conclusion that the "surface area" method is the more efficient and practical basis for the prediction and study of metabolism.

*The influence of temperature changes at the carotid body upon breathing.* THEODORE BERNTHAL and WILLIAM F. WEEKS (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

Warming the blood perfusing the vascularly isolated carotid body region of dogs (carotid sinus excluded) gives rise to increased amplitude and rate of breathing. Conversely, cooling of the perfusing blood is attended by diminished amplitude and rate of breathing. Sectioning Hering's nerve removes these effects.

*Heparin and the formation of white thrombi.* C. H. BEST and CAMPBELL COWAN (by invitation). School of Hygiene, University of Toronto. (Motion picture.)

The first reel consists of a record on panchromatic film of the passage

[1] Am. J. Dis. Children **53:** 273, 1937.

of platelets along a small groove made in a glass slide. The plasma containing the platelets has been heparinized to a slight extent. The other blood cells have been removed by centrifugation. The pictures are taken with the maximum magnification obtainable with an oil immersion lens. The film illustrates the sticking of the platelets to the glass surface, the formation of the digitations, the breaking off of the digitations and various other phenomena.

The second reel shows the formation of white thrombi in a glass cell, inserted as an arterio-venous shunt in the anesthetized monkey, when no heparin is added to the blood. The growth of the thrombi down stream is well illustrated and also the processes by which the masses of platelets break off to form emboli. When heparin is administered no deposition of platelets can be observed. This second reel is made with "kodachrome" film.

*Gastric emptying as influenced by benzedrine sulfate.* KARL H. BEYER (introduced by W. J. Meek). University of Wisconsin, Madison.

In a previous article (Proc. Soc. Exper. Biol. and Med. **37:** 74, 1937) we reported that benzedrine sulfate markedly decreases the initial emptying time of the stomach, but increases the time for final emptying.

The effect of benzedrine sulfate (10–30 mgm. orally) on stomach activity of the normal dog was recorded by the balloon-water manometer-tambour method. It was found that the initial effect of the agent was one of increased rate of activity and increased tonus. The secondary effect was inhibition of contraction, tonus returning to the previous condition. In some instances this inhibition was complete. The first effect occurred usually in about 8 minutes; the latter within 40 minutes.

Pyloric activity under nembutal anesthesia or following decerebration was recorded by a pylorograph-water manometer-tambour system. Intravenous injection of the drug (5–10 mgm.) inhibited activity and slightly increased tonus of the sphincter. This effect lasted for about 5 minutes at which time blood pressure was still maximum or beginning to fall. The pylorus then returned to its former tonus and activity. This subsequent activity was generally slower, more regular, and of greater amplitude.

No constant effect of the drug even in toxic doses on intestinal fistulae was demonstrable.

The increase in initial emptying of the normal stomach is probably due to the primary increase in tonus and activity of the stomach increasing intra-gastric pressure and forcing the contents through an inhibited pylorus whose tonus may be slightly increased. Following the secondary inhibition gastric activity does not return to normal for over an hour, as a rule. The return of pyloric rhythmicity to normal during the gastric depression makes possible further emptying during this period and thus prevents prolonging final emptying of the stomach to an extent undesirable, clinically.

*Modification of latent addition and recovery processes in nerve through continuous polarization.* EDGAR A. BLAIR. Physiological Department, Washington University School of Medicine, St. Louis.

When a conditioning response is blocked at the anode of a constant current, a second response following within 100 milliseconds (msec.) may be propagated. This temporal summation has been regarded as a long

latent addition effect of the initial action potential. At the cathode, an initial response may be conducted, but a second response within roughly the same time may fail to pass. This block has been regarded as indicating a delayed recovery from the initial response (Blair and Erlanger: Am. J. Physiol. **117:** 355, 1936). Since these time values are much greater than any previously reported for nerve, a study was made of latent addition and of refractoriness as affected by the passage of an electric current. Data were secured from individual axons in excised phalangeal preparations of *Rana pipiens* using the amplified spike, recorded by the electron oscillograph, as an all-or-nothing index of response.

At the anode, a subthreshold induction shock can lower the threshold for a period greater than 50 msec., recovery following a response is hastened, and the relatively refractory phase is almost completely eliminated by the development of supernormality which reduces the threshold to about 80 per cent of the resting value.

At the cathode, the lowered threshold due to a subthreshold shock is succeeded in less than 0.5 msec. by an elevated threshold which is referable to the production of cathodal depression by the shock. As cathodal depression dies away, the threshold may again become less than normal, presenting the second phase of increased irritability independently studied by Gasser (This Journal **121:** 193, 1938) in fatigued nerve. Following a response, the refractory phase is greatly increased and supernormality may be completely suppressed.

The duration of the latent addition period following short shocks is of the same order of magnitude in anodally polarized nerves as the duration of temporal summation. The blocking of a second response at the cathode is due to the prolonged refractory period following the conditioning conducted response.

*Potentials from the medulla of the cat.* HELEN BLAKE and MARCEL MONNIER (introduced by R. W. Gerard). Department of Physiology, University of Chicago, Chicago.

Brain potentials from the medulla of the cat, localized with the Horsley Clark instrument and controlled histologically, were picked up with monopolar and bipolar electrodes, amplified by a push-pull amplifier and fed into a crystograph, loud speaker and cathode ray oscillograph.

Spontaneous potentials and those elicited by changing blood pressure and respiration were recorded and the latter correlated with potentials of respiratory muscles and heart beat picked up by electrodes on the chest and with mechanical records of blood pressure and chest movement.

Spontaneous potentials ranged in frequency from 1/sec. to several hundred/sec. and in intensity from 1 to 100 $\mu$ v. The slow potentials are characteristic of grey matter, the fast ones of white. Regular rhythms at 6–16/sec. (but constant for each animal) were found about the midline in the anterior part of the medulla and more laterally in the posterior. Widely spaced monopolar electrodes seemed to pick up these slow waves more readily than closely spaced bipolar ones.

Curare (and artificial respiration) was used to eliminate artifacts from muscles, especially those of respiration, and no change in spontaneous rhythms of the medulla could be detected from the light doses used. Asphyxia produced a high frequency discharge, increasing over several minutes, originating widely through the brain, and breaking up any

regular potentials present. The type of spontaneous discharge can change markedly in one region over a several-hour period, so it is important to consider temporal changes in characterizing the potentials of a region. In the reticular formation, stimulation of which gave blood pressure and respiratory changes, regular slow double rhythms occur, possibly related to heart beat and respiration.

Potentials synchronous with respiration were fairly well localized and especially evident in the reticular formation and dorsal vagus nucleus. Inspiratory potentials were often discrete from expiratory in pattern, location, and time. Inflation of the lungs was found to be more efficient in eliciting respiratory potentials than stimulation of the vagus electrically.

Certain localized regions in the reticular formation, which when stimulated give a rise in blood pressure, yielded potentials which increased with raised blood pressure (adrenalin) and which diminished during asphyxia.

*The occurrence o acetylcholine in gastric juice.* E. BLOCH (by invitation) and H. NECHELES. Department of Gastro-Intestinal Research, Michael Reese Hospital, Chicago, and Department of Physiology, The University of Chicago.

Gastric juice of man and dog was found to contain acetylcholine regularly. An acetone extract of concentrated juice was assayed on the guinea pigs' ileum, dogs', cats' and rabbits' blood pressure and on the leech. These extracts also contain another substance which contracts a strip of intestine and lowers the blood pressure after atropine; this factor seems to be Euler and Gaddum's substance P. Finally, a third toxic substance was found in the extracts which relaxes the intestinal strip and the leech and eventually renders them irresponsive to drugs.

*Blood sugar regulation in animals after adrenal inactivation and liver denervation.* R. C. BODO, S. B. BARKER (by invitation) and A. E. BENAGLIA (by invitation). Department of Pharmacology, New York University College of Medicine and Department of Physiology, Cornell University Medical College.

Animals after adrenal inactivation and liver denervation maintain a normal blood sugar level not only at rest but even during exercise. Evidence is presented that these animals utilize large amounts of carbohydrate and maintain their blood sugar without the aid of adrenine or liver nerves.

Dogs, in which the right adrenal had been removed, the left adrenal denervated and demedullated and the liver nerves severed,[1] after having been kept on a standard carbohydrate rich diet, were exercised during the postabsorptive state on a treadmill for three hours at four miles per hour.

Blood sugar and lactate showed hardly any change during the course of the exercise. The R. Q. varied from 0.86 to 0.91 during the basal period, from 0.77 to 0.81 during exercise and from 0.74 to 0.80 during recovery. The heat production, calculated from the oxygen consumption, increased about four times during the exercise period and returned approximately to the basal figure during recovery.

Calculating the total carbohydrate oxidation from these figures it is evident that these animals are able to utilize large quantities of carbohydrate—in fact, a great part of their total body carbohydrate stores.

[1] See Technique—J. Pharmacol. and Exper. Therap. **62**: 90, 1938.

If liver glycogen is the source of the carbohydrate utilized it can be concluded that mobilization of liver glycogen to maintain a normal blood sugar can occur without adrenine and liver nerves. Experiments now in progress, in which glycogen will be determined in liver and muscle samples taken before and after exercise, are expected to give conclusive evidence as to the source of the oxidized carbohydrate. Similar experiments on completely sympathectomized animals would throw light on the rôle of sympathin.

*Experimental tryptic ulcer of stomach and duodenum.* W. N. Boldyreff (introduced by W. R. Bloor). Battle Creek Sanitarium, Mich.

The round ulcer occurs more frequently in the duodenum than in the stomach. The gastric juice in the duodenum cannot be instrumental in the causation of ulcers as its activity is interfered with by the alkali of the pancreatic juice through neutralization of the acid, and by the bile which greatly inhibits the digestive property of the pepsin. The most destructive factor in the causation of the round ulcer in the duodenum is the pancreatic juice. It not only flows into the deuoenum but is also frequently regurgitated into the stomach where it is capable of inducing ulcers.

Particularly dangerous from the standpoint of ulcer formation is the periodically secreted pancreatic juice which has a very high content of all its enzymes in an active form. This secretion is always accompanied by bile and intestinal juice. During digestion food binds all digestive enzymes and the gastric and the pancreatic secretions to a certain degree neutralize each other. At the time when periodic pancreatic secretion takes place neither food nor the gastric juice are present, and there is nothing to prevent it from manifesting its destructive properties. Once formed the ulcer is maintained not only through the action of the pancreatic juice which caused it, but also through irritation by the gastric juice.

We were never able to induce an artificial ulcer on the skin or in the stomach in animals by means of gastric juice. With the use of pancreatic juice of high enzymic content we observed in all cases in about a week the formation of ulcers of the skin or of the gastric mucous membrane. Neither the size ($\frac{1}{2}$ to $1\frac{1}{2}$ cm.) nor the shape of the ulcers differed in any way from the naturally developed round ulcers of the digestive tract.

Experiments were made on dogs, hens, and pigeons, using canine gastric pancreatic and also duocenal juices.

*Summation of facilitating and inhibitory effects at the neuromuscular junction.* T. E. Boyd, John J. Brosnan (by invitation) and C. A. Maaske (by invitation). Department of Physiology and Pharmacology, Loyola University School of Medicine Chicago.

Responses of the tibialis anticus muscle to stimulation of its motor nerve were observed during progressive intravenous administration of solutions of $MgSO_4$ and $MgCl_2$ to dogs and cats under nembutal anesthesia. When single stimuli are ineffective a tetanus can still be elicited (confirming Lubinska). This tetanus has two components, *a*, an initial peak comparable to that seen in incomplete curarization; *b*, a gradual and sustained secondary rise. These tend to fuse into a normal tetanus after small doses of eserine or on increasing the frequency of stimulation. With increasing dosage of Mg the initial response is gradually extinguished, but its time course meanwhile remains unaltered. The secondary rise persists

longer, but becomes more gradual so that 10 seconds may be required to reach maximum tension. Post-tetanic return of twitch responses is maximum immediately after cessation of the tetanus, and cannot be obtained after the secondary tetanic response is suppressed.

This behavior seems to indicate the existence of two distinct facilitating processes. The first ($F_1$) is rapidly summated and exhausted, the second ($F_2$) is slower in development and disappearance. The same processes probably are responsible for the two phases of facilitation (initial and post-tetanic) demonstrable under curare (cf. Rosenblueth and Morison, Am. J. Physiol. **119:** 236, 1937). Under curare, however, the secondary tetanic response is absent and the post-tetanic recovery appears only after an interval of rest. This is due to a summated inhibitory process which develops concurrently with and masks $F_2$, but subsides more rapidly than the latter. Post-tetanic recovery is temporarily suppressed during, but prolonged by, a second period of rapid stimulation of the nerve. Under Mg the masking of $F_2$ does not occur, the inhibitory effect being absent or negligible.

*Excitability and conduction in visceral muscle.* EMIL BOZLER. The Ohio State University, Columbus.

Under suitable conditions electric stimulation of uterine muscle and the ureter produces a propagated contraction. These muscles behave as if they were large single muscle cells and their properties can be explained by assuming syncytial connections between the muscle fibers. The propagation of an impulse in smooth muscle is accompanied by an action potential which, except for its slowness, is not different from that of nerve. In the ureter the spike potential is followed by a strong positive after-potential. The spontaneous contractions of uterine muscle are tetanic contractions produced by a repetitive discharge at a frequency of 1 to 2 impulses per second.

The time relations of the electric responses as well as other characteristics of excitability, chronaxie, refractory phase and velocity of conduction, vary widely in different species and organs, and change under certain physiological conditions. The characteristics of various smooth muscles can be grouped in an almost continuous series with those of nerve fibers, suggesting that the excitatory processes differ only quantitatively in the two kinds of tissues.

*The effect of cinchophen and dehydrocholic acid on bile secretion.* W. B. BRADLEY (introduced by C. A. Dragstedt). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

The cystic ducts were ligated and the common bile ducts cannulated in ten dogs anesthetized with pentabarbital. The normal rate of flow, bile pigment, bile salts, and cholesterol content of the bile were determined for each dog. The sodium salts of cinchophen and dehydrocholic acid were then administered intravenously in doses of 50 mgm./kilo. Cinchophen increased the flow 510 per cent during the first hour, this increase diminishing to 300 per cent by the fifth hour. In no dog did the rate of secretion return to the control level before the completion of the experiment. However, with dehydrocholic acid, the flow increased 650 per cent in the first hour but rapidly returned to the control level during the third hour. Cinchophen increased the excretion of bile pigment and cholesterol and

this increase, though greatest during the first hour, was maintained throughout the experiment. Bile acid secretion rapidly decreased after cinchophen in some cases disappearing from the bile completely. Dehydrocholic acid caused an initial increase in bile pigment, bile salts, and cholesterol, which increase was followed by a compensatory decrease. Cinchophen was found in the bile in large quantities, 20 per cent of the administered dose being excreted through this channel in 5 hours.

*A study of the excretion of urinary ammonia and phosphate, as correlated with changes in urinary and blood pH produced by variations in pulmonary ventilation and chemical injection.* CHARLES R. BRASSFIELD and VIVIAN G. BEHRMAN (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

Through the use of a glass electrode arrangement, the pH of arterial blood and urine was recorded continuously in anesthetized dogs under chemical injection, high carbon dioxide and low oxygen administration. Uretral catheters were placed in both ureters, one being connected to the glass electrode and the other to the drop recorder. The urine flowing through the electrode was collected under toluol in 1 cc. samples. Microdeterminations for ammonia nitrogen and inorganic phosphate were made on these samples. Conway's micro-ammonia method (1933) and Kuttner and Lichtenstein's modification of the Bell-Doisy colorimetric method for inorganic phosphate (1930) were used.

Acid phosphate injection caused only a slight decrease in the blood pH, whereas, the urine exhibited a more pronounced decrease in pH. The rise in phosphate excretion paralleled the urinary pH decrease. Ammonia excretion, however, was unchanged by phosphate injection. Disodium phosphate injection, in like manner, increased the phosphate excretion, but seemed to have no effect on ammonia formation.

Carbon dioxide administrations (12–15 per cent) produced a sudden decrease in blood pH accompanied by a gradual decrease in urinary pH. The urinary phosphate increased during the hypercapnia. In cases where the urinary flow was markedly decreased, the phosphate increase as well as the pH decrease occurred at the close of the administration. In four out of six procedures, ammonia excretion increased as the urinary pH decreased. The remaining two instances showed a decreased urinary ammonia at the onset of $CO_2$ administration, followed by no change or a slight increase.

Low oxygen (8–10 per cent) produced an immediate increase in blood pH accompanying increased ventilation whereas the urine shows a gradual increase in pH. Ammonia determinations showed either no change or a decrease in ammonia excretion.

*The influence of sex and age on postural stability.* GENEVIEVE L. BRAUN (by invitation) and FRANCES A. HELLEBRANDT. University of Wisconsin, Madison. (Read by title.)

The disproportionate height of the center of gravity of the human body in the vertical posture and the smallness of its base of support are mechanical factors in build conducive to static insecurity. The integrity of the myotatic reflex is fundamental to the maintenance of a stable upright stance. The effective stimulus for the postural contraction of the antigravity muscles is stretch and the receptors involved are the proprioceptive

end-organs indigenous to the muscles primarily concerned. Since the posterior columns, the spinocerebellar and corticospinal pathways are among the earliest fiber tracts to undergo myelination, the child should be at no neurological disadvantage in the performance of this neuromuscular act.

The incessant shifting of the center of weight which characterizes the standing of man was studied in 111 subjects, 43 male and 68 female, ranging in age from the first to the eighth decade. The method, which has already been described (Hellebrandt, Am. J. Physiol. **121:** 471, 1938), permitted the determination of the mean position of the center of gravity, its maximal shift in the coronal and sagittal planes, and the projection of these points into the base of support. The area of maximal sway was greatest in the first decade, decreased during the second, and posture reached its peak stability in the third. The mean sway increased in the middle aged and again in those above 50. But more striking than this relatively slight variability is the fact that the average area of maximal sway for the group as a whole was only 4.09 sq. cm., the center of weight oscillating within a region equivalent to but 1.27 per cent of the mean base of active support and occupying a position remarkably close to the geometric center of the area of underpropping with an ample margin of static security in all diameters of the anatomical base of support. Sex differences were not statistically significant. The eccentricity of the mean center of weight was slight, in the majority, at all ages and in both sexes, falling forward and to the left of the geometric center of the base.

*Chemically induced rhythmicity in peripheral axones.* FRANK BRINK, JR. (by invitation), T. SJOSTRAND (by invitation) and D. W. BRONK. Johnson Foundation, University of Pennsylvania, Philadelphia.

Nervous tissue shows two well defined types of rhythmicity. There is the discharge of impulses which starts at a high frequency and then falls to some constant low value. This is characteristic of the discharge in the axone when stimulation occurs through an end organ. The second type of rhythmicity is the grouping of bursts of activity where each burst shows a graded frequency of impulses. This type occurs in the central nervous system and is well illustrated by discharges from respiratory motoneurones. In the present experiments we have endeavored to determine whether these modes of action are inherent in peripheral axones where the activity can be more directly analyzed.

By soaking a single axone in sugar solution or in isotonic NaCl the organization of its irritable structure is so altered that it responds to application of sodium citrate with a discharge of impulses which starts at a high frequency and falls to a sustained low frequency. This is analogous to the discharge in axones from tension receptors when a constant stimulus is suddenly applied. If the stimulating solution be sodium chloride plus 0.02 per cent KCl, the bursts are similar but shorter, and their length can be further shortened by adding $CaCl_2$. These discharges are like those from rapidly adapting end organs.

An axone can be excited to intense activity by removal of calcium. If Ringer's fluid is applied during such activity the discharge may break up into groups of impulses, each group having the characteristics of the bursts described above. Furthermore, the duration of the groups and of

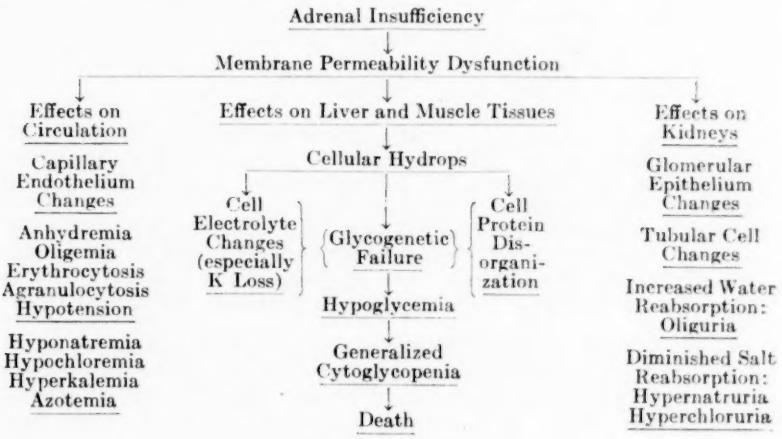
the intervals run a definite time course. The longer the duration of the group the longer the interval except near the end of activity when the intervals lengthen with no correspondng increase in the duration of the bursts.

*A possible interpretation of cortico-adrenal function.* S. W. BRITTON. Physiological Laboratory, University of Virginia. (Read by title.)

Animals suffering from adrenal insufficiency show a number of diverse and serious disturbances which may be explicable on a fundamental basis. The renal and circulatory changes, as well as the carbohydrate effects, are possibly to be referred to primary dysfunction in permeability relationships in the organism. A good deal of experimental evidence supports this conception.

Potassium appears to be highly important in maintaining the protoplasmic complex in cells, and in its absence rapid proteolysis occurs. There are also found wide disturbances in carbohydrate metabolism in the body in adrenal insufficiency which appear to be interrelated with potassium concentration in blood and tissues. The glucose content of red blood cells and glycogen levels in muscle and hepatic tissues are not normally maintained in the absence of the adrenal cortex. Anhydremia, hypertension and other circulatory changes, and disturbances in reno-secretory function, would appear further to have a single underlying etiological factor. These conditions along with recognized variations in potassium and sodium and chloride balance in adrenal insufficiency indicate a basic disorganization in the activities of semipermeable membranes.

It might be proposed as a general hypothesis that the adrenal cortex is responsible for the maintenance of balanced relationships between the materials within and those surrounding the cells in the body. Possibly the lipoid complex of cell membranes is preserved by the cortico-adrenal hormone. Certainly there are many indications that deterioration of the cellular envelope occurs in the absence of the adrenal glands.



A scheme showing the probable progressive changes in adrenal insufficiency, in keeping with most of the experimental evidence, has been devised. Glycogenetic failure and thus hypoglycemia and possibly generalized cytoglycopenia appear to represent the most serious events which lead to death after adrenal removal.

*Unconditioned stimulus substitution in the conditioning process.* W. J. BROGDEN (introduced by W. Horsley Gantt). Pavlovian Laboratory, Phipps Psychiatric Clinic, Johns Hopkins University.

The unconditioned stimulus in any conditioning process performs a dual function: 1, it determines the form of response; 2, it provides incentive for that response. The conditioned stimulus can evoke the response, but cannot maintain it without the presence of the unconditioned stimulus. It should be possible, however, to evoke a consistent response to the conditioned stimulus, by substituting a reinforcing agent for the unconditioned stimulus. Furthermore, if this agent produces a different response, the conditioned stimulus should call it forth in addition to the original response.

To test this hypothesis, forelimb retraction from a charged grid was conditioned to bell in three dogs. When the animals scored 100 per cent response, the shock was omitted and each successful trial was reinforced with food. Two animals, instead of retracting to bell, turned immediately to the food-box. After being returned to bell-shock sequence to re-establish forelimb retraction, one dog maintained the limb response reinforced by food; the other had to be returned three times before attaining a similar level. The three animals gave a mean response of 98 per cent for 1000 trials with food reinforcement, compared to 99 per cent for a control group of three animals with shock reinforcement. The salivary response, even though reinforced with food 98 per cent of the time, was irregular in both magnitude and appearance, the mean being 65 per cent for 1000 trials.

Of the two conditioned responses initiated by bell, only the retraction response was regular and frequent, although reinforced by food which never evoked it. The salivary response, both motivated and evoked by food, appeared infrequently and irregularly to bell in spite of almost constant reinforcement. Prior training of the retraction response together with its obligatory appearance for food reinforcement, probably accounts for its dominance over the salivary response.

*The influence of altered chemical environment on the activity of ganglion cells.* D. W. BRONK, M. G. LARRABEE (by invitation), J. B. GAYLOR (by invitation), and F. BRINK, JR. (by invitation). Johnson Foundation, University of Pennsylvania, Philadelphia.

The perfused stellate ganglion has been used for studying the effects of various chemical agents on the activity of nerve cells and on the mechanism of synaptic transmission. The response of the ganglion cells was determined by recording their discharge of impulses into the postganglionic nerve.

Oxygen lack due to perfusion with de-oxygenated Ringer's fluid or arrested circulation causes the progressive and reversible failure of ganglionic pathways (Bronk and Larrabee, Am. J. Physiol. **119:** 279, 1937; Bargeton, Ibid. **121:** 261, 1938). Such recovery can follow periods of oxygen lack exceeding three hours. The rate of failure is greatly increased by moderate

activity for stimulation at 9 per second blocks conduction in all fibers within 10 minutes. If the stimulus frequency is then reduced some cells again respond for a short time. At low temperatures (12°) the anoxic ganglion conducts for more than 6 hours.

The rate of failure of synaptic pathways deprived of oxygen is not significantly different from that of some axones. For transmission of impulses directly through the ganglion into the cervical sympathetic nerve fails as rapidly as synaptic transmission into the inferior cardiac nerve.

Acetylcholine or potassium chloride injected into the perfusion fluid stimulates ganglion cells directly. We have used this property to determine what portion of the ganglionic pathway is most susceptible to oxygen lack. While observing progressive failure of synaptic transmission these chemicals have been injected from time to time. Failure of the postganglionic discharge to chemical stimulation and to preganglionic stimulation runs the same time course; the time of complete failure is the same for either type of stimulus. We therefore conclude that the postsynaptic unit fails as soon, if not sooner, than the synaptic mechanism.

An increase of calcium blocks transmission. If the calcium be reduced by perfusion with sodium citrate, on the other hand, the cells discharge spontaneously at a time when synaptic transmission is blocked. In several experiments we have observed, during the early stages of low calcium, the development of repetitive after-discharge following each preganglionic volley.

*The effect of transection of the pituitary stalk on the F.S.H. and L.H. content of the rabbit's pituitary.*[1] CHANDLER MCCUSKEY BROOKS. Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, Md.

It has been stated previously (Brooks, 1937, 1938) that coitus does not produce ovulation in the rabbit after section of the pituitary stalk. Failure of the pituitary glands of such animals to discharge ovulation-producing material following coitus might be due to an inability of the anterior lobe to manufacture the necessary gonadotropic hormones. In order to determine the gonadotropic hormone content of such glands the pituitaries of fourteen stalk-cut rabbits were assayed; five for F.S.H., three for L.H. and six glands were divided, one part being tested for F.S.H. and the remaining portion of each being assayed for L.H.

The mouse uterus test as described by Levin and Tyndale (1937) was used in the F.S.H. assays. The rabbit ovulation test for L.H. was employed in three cases and the production of premature ovulation in mice was used as a criterion of L.H. in the remaining six instances. The glands of a similar number of normal animals were assayed in the same way.

It was found that the glands of two of the stalk-cut rabbits were deficient in their gonadotropic hormone content. Five glands contained abnormally high concentrations of both F.S.H. and L.H. and the other seven glands were normal. It can be definitely stated that the failure of stalk-cut rabbits to ovulate following coitus is not due to an inability of the pituitary to maintain a normal content of F.S.H. and L.H.

[1] This work was supported by a grant (to Dr. Philip Bard) from the Committee for Research in Problems of Sex, National Research Council.

*Effect of theelin and progestin upon proliferation of skin in the mouse.* W. R. BROWN (by invitation), HARLOW ADES (by invitation), and FRED A. METTLER. Departments of Biochemistry and Anatomy, University of Georgia School of Medicine, and Department of Zoology, University of Illinois.

Young adult male mice were treated with large doses of theelin and crude corpus luteum extract. The number of mitotic figures in the skin was taken as an index of the effect of the injected substance upon skin proliferation.

The corpus luteum extract in all cases produced a marked decrease in the number of mitotic figures in the animals studied over the control group. The effect of theelin was less decisive, causing a marked increase in the mitotic figures in one experiment, and a decrease in two subsequent experiments. In no case, however, was the decrease due to theelin as great as that due to the corpus luteum extract.

*The effect of intramuscular injection of gonadotropic substances on the corpus luteum phase of the human menstrual cycle.* J. S. L. BROWNE and ELEANOR H. VENNING (by invitation). McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

The effect of gonadotropic extracts from human pregnancy urine (Physex) and from the serum of pregnant mares (Antex) on the luteal phase has been studied in seven cases of functional sterility in whom periodic bleeding was occurring at more or less regular intervals. The excretion of pregnanediol glucuronidate in the urine was used as an index of progesterone production. As has been previously reported pregnanediol glucuronidate is excreted in smaller amounts and over a shorter period than in the normal individual in many of these cases. The extracts were in the form of dried powder and were injected intramuscularly in physiological saline; both types were labelled 100 mouse units per ampoule. The pregnancy urine extract assayed at 300 and the mare's serum extract at 150 units per ampoule using a positive vaginal smear in the immature rat as the criterion and the pregnancy urine extract showed 170 rat luteinizing units per ampoule. The results varied and depended upon whether the injections were made before or after the beginning of the luteal phase. If either type of extract was injected prior to the beginning of the luteal phase, this phase tended to appear at an earlier time in the cycle and depending upon the length of this luteal phase the total length of the cycle might or might not be shortened. On the other hand if injections of pregnancy urine extract (100 mouse units daily) were given after the luteal phase had begun the total amount of pregnanediol excreted was increased and the luteal phase could be extended to as long as 17 days, the onset of the next bleeding being correspondingly delayed. It is doubtful how much longer the luteal phase could be maintained with this dose since the rate of excretion of pregnanediol tended to decrease towards the end of this time. The pregnanediol excretion generally ceased on the second day after the injections were stopped and bleeding began on the third day.

These extracts are then capable of affecting the function of the human ovary in this type of case when used in moderate doses given intramuscularly.

*Further study on electrical polarity in the animal in relation to anesthesia and unconsciousness.* W. E. BURGE. Department of Physiology, University of Illinois, Urbana.

Immediately upon connecting the galvanometer with the gastrocnemius muscle of a medium sized frog, a current of 6.5 micro-amperes flowed from the uninjured to the injured portion of the muscle. After one minute, the current had decreased to 5 micro-amperes, after two minutes, to 4.5 micro-amperes, after three minutes, to 4.3 micro-amperes, after four minutes, to 4.2 micro-amperes, and so on until after three hundred and sixty minutes, to 0.3 micro-amperes. Similarly when a 1.5 volt dry cell was connected with an ammeter, 15 amperes of current flowed immediately. After one minute the current had decreased to 12.4 amperes, after two minutes, to 11.8 amperes, after three minutes, to 11.2 amperes, after four minutes, to 10.6 amperes, and so on until after three hundred and sixty minutes, to 0.4 ampere.

Hence, when the injured, negative, portion of the muscle was connected by a wire to the uninjured, positive, portion, electrons, or negative charges, passed from the negative, injured portion to the positive, uninjured portion of the muscle until the two parts were of equal potential, just as was the case when the positive and negative poles of the battery were connected.

A medium sized goldfish was placed in a glass vessel in water, between two platinum disc electrodes. When the head of the fish was towards one electrode, the beam of light swung across the galvanometer scale to one side and, upon the fish reversing its position the beam of light swung to the other side of the scale. Hence the fish, like the injured muscle and battery, exhibited polarity with its head region one pole, and tail region the other pole.

When the fish came to rest for any length of time, there was a decrease in the electrical potential which was restored when the fish became active again. Asphyxiation with $CO_2$ decreased the potential and caused a reversal in polarity. Upon recovery from asphyxiation, there was a reversal and return to the original polarity. The volatile anesthetics caused a decrease in potential in the fish similar to what had been observed in the dog (Burge, Wickwire, Orth, Neild, and Elhardt, Am. J. Physiol. **116:** no. 1, 1936).

*The absorption of sorbitol, mannitol, dulcitol, and erythritol by chronic loops of small intestine.* G. E. BURGET, W. R. TODD (by invitation), and H. F. HANEY (by invitation). Departments of Physiology and Biochemistry, University of Oregon Medical School, Portland.

Dogs with chronic closed jejunal loops were used. The loops were attached to the anterior abdominal wall where they could be readily entered with a hypodermic needle (Am. J. Digest. Dis. and Nutrition **1:** 722, 1934). The rates of absorption of the above substances were compared to the rate of absorption of glucose. Mannitol and sorbitol were usually used in 10 per cent solutions while dulcitol, being less soluble, was used in somewhat lower percentages. Depending upon the size of the particular loop, either 10 cc. or 5 cc. quantities were injected. After one hour the loop was aspirated and washed with warm 0.9 per cent NaCl solution. The as-

pirated fluid and washings were mixed and the unabsorbed material determined. Thus the amount absorbed was readily obtained by subtracting amount recovered from the amount injected. Several experiments were run on a given dog for each substance used.

Erythritol was found to be absorbed more readily than glucose when in equal concentrations by weight. In equimolecular concentrations this difference tended to disappear. Sorbitol was taken up slightly less readily than glucose while mannitol and dulcitol were absorbed much more slowly than glucose. If the rate of absorption of glucose be taken as 100, that for mannitol and dulcitol from these observations would be approximately 60. These results are of interest with regard to the mechanism of absorption.

*The vitamins B and insulin tolerance in the albino rat.* J. C. BURKE (introduced by A. R. McIntyre). Department of Physiology and Pharmacology, University of Nebraska Medical College, Omaha.

When the ratio of the heat stable vitamin B substances to thiamine in a diet fed to albino rats is modified the insulin tolerance of the rats is also modified. An increase in the relative proportion of thiamine increases the effect of insulin as measured by the duration of hypoglycemia. The extent of the fall in the blood sugar is not significantly greater. Conversely when the proportion of thiamine in the diet is decreased relative to the heat stable B substances a (autoclaved yeast), b (flavin), c (autoclaved flavin-free rice polishings), the duration of insulin hypoglycemia is also reduced. The addition of large amounts of a, b and c to a normal diet tends to reduce both the degree of insulin hypoglycemia and its duration. Rats deprived of all heat labile and heat stable vitamin B substances become hypersensitive to insulin when they commence to lose weight. Rats fed a diet free of heat stable vitamin B substances but containing thiamine become exceedingly hypersensitive to insulin. The blood sugar of rats, maintained on a diet relatively low in thiamine but rich in autoclaved flavin-free rice polishings returns to its initial (24 hour fasting) level and rises above this level about 4 hours after injection with 2 units of insulin per kilogram. Rats receiving large amounts of thiamine relative to the flavin and "$B_6$" intake exhibit hypoglycemia 6 hours after the injection with 2 units of insulin per kg. Representative blood sugar curves following the injection of (1) insulin and (2) dextrose in rats maintained on various diets will be shown. Liver glycogen studies are now in progress.

*Vitamin C and insulin tolerance in the albino rat.* A. R. MCINTYRE and J. C. BURKE (by invitation). Department of Physiology and Pharmacology, University of Nebraska Medical College, Omaha. (Read by title.)

(1) Rats receiving a thiamine-free diet of commercial casein, starch, lard, salt mixture and cod liver oil were allowed to lose 15 to 19 per cent of their weights and then injected with 20 units of insulin per kilogram body weight per day for five days. All animals were rendered comatose and half the group died after the second or third day. A second group receiving the same diet but supplemented with 0.2 mgm. of cevitamic acid per rat per day were injected with the same amount of insulin daily for five days after they had lost the same proportion of their peak weights. All animals survived without appearance of coma and with only moderate depression. (2) Rats receiving, for 21 days, a B-free basal diet of flavin free casein, sucrose, lard, salts and cod liver oil supplemented with 4

gamma of crystalline $B_1$ and 2 gamma of Lactoflavin per gram of food exhibit a different blood sugar curve after the injection of 2 units of insulin per kilogram (after 24 hours' starvation) than do animals on the same diet with the additional supplement of 0.1 mgm. of cevitamic acid per gram of food. The blood sugar of those animals receiving cevitamic acid returned to the initial level (24 hour fasting level) after 3–4 hours and rose above the initial level after 4–5 hours. In those animals receiving no cevitamic acid the blood sugar did not return to the initial level until after 6 hours. (3) Animals from these two latter groups were subjected to hypoglycemic shock after a 24 hour fast with insulin injections of 4 units per kilogram, followed in one hour by 2 units per kilogram each succeeding hour. Animals receiving the cevitamic acid survived 50 per cent more insulin than did the controls although their blood sugar levels dropped considerably lower as evidenced by hourly analysis. Because of the known hypersensitivity to insulin of rats fed a diet deficient in heat stable vitamin B complex (McIntyre and Burke. Am. J. Physiol. **119:** 364 1937) and the above findings which indicate a protection from insulin shock in such animals when given vitamin C, we make the tentative suggestion that these facts may be related to a disturbance in the "vitamin C" content of rats on a heat stable vitamin B deficient diet.

*The measurement of blood-flow, venous pressure and distensibility of the digital vessels.* ALAN C. BURTON. Johnson Foundation, University of Pennsylvania, Philadelphia.

The well known plethysmographic method in which the rate of increase of volume of a limb is observed when venous return is arrested has been adapted to the measurement of blood flow in the digits. The volume changes of the last two phalanges of a finger or toe is recorded photographically by a mirror manometer while occlusion of the veins is made by a cuff on the remaining phalanx.

Upon occlusion the volume increases to a steady value in a few seconds. From the initial rate of increase the flow in the fingers is found to vary from less than 5 to 60 cc./min./100 cc. of tissue between full constriction and dilation. There is a normal fluctuation of about 30 per cent from moment to moment, partly due to a respiratory effect. Holding the lungs inflated enormously reduces the finger flow.

When the total volume-increase is plotted against the pressure used for occlusion a straight line results. The intercept of this with the pressure axis gives the venous pressure, while its inclination measures the "distensibility" of the vessels. Changes of venous pressure with the level of the hand and with blood flow are shown. The distensibility is less with dilated than with constricted vessels, giving no evidence of "active" tone of the vessels responsible for the finger-volume pulse. The resistance of the tissues seems not to be a limiting factor in the normal, but may be so in some conditions (scleroderma).

When positive pressures are used in the plethysmograph the finger volume pulse increases many times. Its variation with pressure gives information as to arteriolar and capillary pressures.

The method has been used to make a direct correlation between skin temperature, pulse volume and the plethysmographic determination, and for the study of peripheral vascular diseases, where flow determinations on the whole limb may be insensitive. A convenient, portable apparatus has been developed and will be described.

*The experimental production of severe homogeneous osteoporosis by gastrectomy in puppies.* R. A. BUSSABARGER (by invitation), SMITH FREEMAN and A. C. IVY. Northwestern University Medical School, Chicago. (Demonstration.)

The taxidermic specimens of a dog gastrectomized at three months of age and sacrificed at 510 days of age and its litter mate control will be presented. The gastrectomized specimen manifests the deformities of rickets, but its bones did not manifest the characteristics of rickets, because the animal had received ample vitamin D. The bones from these two specimens and also those from other dogs gastrectomized at an early age, and also at a mature age, will be displayed (Am. J. Physiol. **121:** 137, 1938).

*Respiratory modifications of cardiac output.* DANIEL CAHOON (by invitation), VICTOR JOHNSON and JEANNE HOWARD (by invitation). Department of Physiology, University of Chicago.

The stroke volume of barbitalized dogs breathing normally was recorded by means of a cardiometer placed over the heart through an opening in the chest wall, which was subsequently sealed airtight. During inspiration the stroke volume was diminished (as has been reported previously), but (contrary to previous reports) the diastolic size of the ventricles also decreased in inspiration, pointing to a diminished ventricular filling in this phase of respiration.

The chest of the animal was so prepared that the conditions of pneumothorax and of normal respiration could be produced in a few seconds, and alternated at will. Sudden pneumothorax almost completely abolished the changes in stroke volume, which were at once re-instituted by restoration of normal breathing. Since the breathing movements of the chest and diaphragm continued throughout, it was felt certain that the stroke volume changes recorded were real, and not artifacts caused by the chest movements affecting the position of the cardiometer on the heart.

By means of windows in the chest wall, sealed airtight to permit normal respiration, direct visual inspection of the auricles could be made. The right auricle increased appreciably in size in inspiration, expanding much like the adjacent lung tissue. In expiration it suddenly collapsed, accounting (at least in part) for the increased diastolic ventricular size and ventricular stroke volume at that time.

During the exaggerated breathing movements after double vagotomy, the above effects were accentuated.

*Prolonged diabetes after the administration of extracts of the anterior pituitary gland.* JAMES CAMPBELL (by invitation) and C. H. BEST. Department of Physiology, University of Toronto.

After the report by F. G. Young that what appeared to be a permanent diabetes may be produced in dogs by the intraperitoneal injection of certain extracts of the anterior pituitary gland, similar experiments were initiated in this department. Our findings are in accord with those reported by Young. Some of the animals failed to respond, in that diabetes did not appear. In one, a mild but constant glucosuria persisted after discontinuing the injections of the extract; in another, the sugar excretion was most irregular. In a third, an intense glucosuria persisted for a prolonged period and showed no signs of abatement. This animal was no more resistant to insulin than the average depancreatized dog. The findings with regard to the sugar excretion and sensitivity to insulin will be reported in some detail.

*The effect of parathyroid hormone on the diffusibility of calcium into the peritoneum.* A. CANTAROW, V. HAURY (by invitation) and C. G. WHITBECK (by invitation). Departments of Medicine and Pharmacology, Jefferson Medical College, and the Laboratory of Biochemistry, Jefferson Hospital, Philadelphia.

Ten dogs were given dextrose (2.5 per cent) and saline (0.9 per cent) solution intraperitoneally (100 cc. per kgm.). After 16 hours, blood (femoral artery) and peritoneal fluid were withdrawn (20 cc. each) and 500–600 units parathyroid hormone[1] injected. Blood and fluid samples were obtained hourly for 5–8 hours for Ca, P and Mg determinations.

The fluid-serum Ca ratio was 52.3–69 per cent (average 61) in the resting state (16 hours after peritoneal injection). In 8 instances, parathyroid administration was followed by a progressive increase in serum calcium, the maximum increase being 1.8–2.7 mgm. at 5–6 hours. The fluid Ca rose more gradually, the rise continuing throughout the experimental period in all but one case, despite terminal fall in serum Ca. The fluid-serum Ca ratio remained essentially unchanged or decreased slightly during the first 3–6 hours and then increased to a maximum (65–86 per cent) at the end of the experimental period (8 hours).

In 2 animals previously subjected to repeated filling and drainage of the peritoneum, parathyroid administration was followed by a drop in serum Ca to 6.89 and 6.05 mgm. at 3 and 5 hours respectively. In one, the fluid Ca fell after a preliminary increase; in the other it increased progressively. In both, the fluid-serum Ca ratio increased during the first 3–5 hours and then decreased.

If the fluid Ca may be regarded as representing the diffusible fraction of serum Ca, it appears that parathyroid hypercalcemia, under normal conditions, is due to proportionate increase in diffusible and nondiffusible fractions. In only one case was the period of increasing calcemia accompanied by an increased fluid-serum Ca ratio. Findings in 2 cases suggest that if the available calcium reserves are depleted parathyroid hormone may produce hypocalcemia, under the conditions of the experiment, and that the diffusible fraction may be markedly increased at the expense of the nondiffusible. Under these circumstances, the latter may serve as the source of mobilizable diffusible Ca.

*The effects of large doses of pentobarbital sodium (nembutal) on pregnant guinea pigs.* EMMETT B. CARMICHAEL. Department of Physiological Chemistry, School of Medicine, University of Alabama. (Read by title.)

Pregnant guinea pigs that were near full term have been injected intraperitoneally with a fresh aqueous solution of pentobarbital sodium. Seventy-seven pigs were employed and their weights varied from 460 grams to 920 grams. The doses varied by increments of 2.5 mgm. from 35 mgm. to 47.5 mgm. per kilogram body weight of guinea pig.

There was an increase in both the maximal hypnotic time and the per cent of mortality over that of males and non-pregnant females of approximately the same weight for doses of 42.5 mgm., 45 mgm., and 47.5 mgm. of the drug per kilogram. About 20 per cent of the pregnant females delivered within 24 hours after the injection of the drug and a majority of these young pigs were definitely premature at birth as evidenced by their weights, the length of their hair, the length of time after birth be-

[1] Parathyroid hormone was obtained through the courtesy of Dr. E. A. Sharp of Parke, Davis & Co.

fore they were able to stand, their gait, and by the fact that several survived only a day or two.

*Effects of hexoses upon the respiratory quotient of the rhesus monkey.* THORNE M. CARPENTER and CARL G. HARTMAN. Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Mass., and Department of Embryology of the Carnegie Institution of Washington, Baltimore, Md.

Observations were made with rhesus monkeys given 14 grams of glucose, fructose, or galactose. The measurements began within 20 minutes after introduction of sugar by stomach tube and continued in half-hour periods for 4 hours. The respiratory quotient was determined by the open-circuit method. In control experiments with fasting monkeys not previously handled the maximum quotient was not obtained until the fourth half hour. Control experiments with 105 cc. of water showed that the quotients in at least the first two half hours were lower than in the remaining periods. The approximate average quotient after 24 hours' fasting was 0.75. This served as a base-line for calculation of the rise in quotient in the sugar experiments.

Ingestion of glucose produced a sharp rise in the quotient, beginning in the first half hour. The maximum average was 0.97 in the fourth half hour. At the end of the series the average was 0.82. Fructose resulted in a maximum average of 0.87 in the third half hour and a return to 0.76 at the end of the fourth hour.

Galactose caused but a small rise, the maximum quotient being 0.82, which persisted from the third through the seventh half hour. In two instances urines voided during or immediately after the experiment contained over 3 grams of sugar calculated as galactose. The monkey apparently has a low tolerance for galactose, and there is little indication that much of it is burned.

This is another contribution to studies of biological variations in the effects of ingestion of hexoses upon the respiratory quotient. After glucose the respiratory quotient of the monkey rises more rapidly and higher and decreases more rapidly than that of man. The monkey burns glucose more rapidly but probably stores less as glycogen. The metabolism of fructose is about the same with both. After galactose the rise in quotient is not so great as with man but more like that in unpublished experiments with rats.

*Urinary excretion of follicle stimulating hormone in young and adolescent boys.* H. R. CATCHPOLE, W. W. GREULICH and R. T. SOLLENBERGER (introduced by J. F. Fulton). Laboratory of Physiology and Departments of Anatomy and Psychology, Yale University, New Haven.

Hormone was recovered from the 24-hour urine specimen by tungstic acid precipitation followed by acetone-ammonia extraction. Assays were made by the technique of Levin and Tyndale (Am. J. Physiol. **119:** 360, 1937) using the 21–22 day old female mouse and observing the uterine weight at 72 hours after injection of the hormone in 6 equal doses over 48 hours. Standardization curves have been made by plotting graded doses of standard gonadotropic preparations (pregnant mare serum, castrate urine) against the ratio Uterine weight/Body weight, and the mouse unit taken as the smallest amount of hormone causing a 100 per cent increase in this value over the control ratio. As little as 2 mouse units per 24 hours is detectable.

Determinations were made on 33 boys of prepuberal and adolescent status. Eighteen boys aged 11 and under, all falling into a frankly immature class showed no excretion of follicle stimulating hormone (less than 2 MU per 24 hours). Of 15 boys in the age group 12 to 16, four showed absence of hormone, and twelve amounts varying from 2 to 10 units, the latter being an essentially adult figure. The actual amounts found over this period are related to developmental status rather than to the chronological age.

*The endocrine control of the blood lipids of the bird.* I. L. CHAIKOFF, C. ENTENMAN (by invitation) and F. W. LORENZ (by invitation). Division of Physiology of the Medical School and the Division of Poultry Husbandry of the College of Agriculture, University of California, Berkeley.

In previous reports from this laboratory it was shown that an increase in the lipid content of blood and liver occurs in the bird during laying. Ovarian activity is apparently involved in these lipid changes, for a rise in blood lipids was first noted in the pubescent female bird containing rapidly growing yolks and at no time occurred in the male bird from early age to maturity.

In order to throw light on the mechanism of the increased lipid metabolism found in the laying state, an investigation was made of the effects of hormones that stimulate ovarian activity. In the present report the effects of the gonad-stimulating hormone from pregnant-mare serum are recorded.

Nine immature Single-Comb White Leghorn birds, 45 to 55 days old, received daily intramuscular injections of pregnant-mare serum for periods of 3–4 weeks, each bird receiving a total of 900 rat units per week. Average blood lipid values obtained before and at the end of the injection period are shown below:

*Lipid values expressed in milligrams per cent of whole blood*

| | CHOLESTEROL | | | TOTAL FATTY ACIDS | PHOSPHOLIPID | TOTAL LIPID |
|---|---|---|---|---|---|---|
| | Total | Free | Ester | | | |
| Initial | 110 | 86 | 24 | 335 | 300 | 445 |
| Final | 163 | 122 | 41 | 945 | 608 | 1104 |

A rise in the blood lipid level was obtained in birds injected with pregnant-mare serum. All lipid constituents responded but the most pronounced rise occurred in neutral fat. Considerable irregularity in the lipid response to the hormone was observed, and this in part paralleled an irregularity of response in the secondary sex characters. Since no yolk growth had occurred at the time that the blood was examined for lipids, it would seem that a rapid passage of fat into the egg is not necessary to initiate the rise in blood lipids in the pubescent or laying state.

*The relation between serological specificity and molecular orientation at cell surfaces.* L. A. CHAMBERS (introduced by D. W. Bronk). Johnson Foundation, University of Pennsylvania, Philadelphia.

An approach to the general problem of the specific behavior of cell surfaces with particular reference to the rôle of molecular orientation has been made by comparing the specific response of oriented monolayers of surface materials with the known properties of the intact cell. Excellent

material for such study is the so-called labile antigen extracted from Strep. hemolyticus. Monofilms of this complex protein were deposited on prepared glass slides in such a way that a hydrophobic surface was exposed in some cases and a hydrophilic surface in others. Such monolayer preparations were agglutinated with homologous living organisms by the addition of both heterologous and homologous antisera. The number of bacteria adhering in each case was estimated by direct count after staining with methylene blue. The data so obtained show that the hydrophobic surface responds only to the type specific antibody while the hydrophilic surface gives a marked nonspecific antigen-antibody reaction. From this evidence one may conclude that the hydrophobic orientation is most probable in the cell surface since the nonspecific reaction of the hydrophilic groups conforms with the observations of Mudd and his co-workers that the labile antigen after extraction contains a nonspecific component which is not apparent so long as the organisms are intact. Thus data obtained by modification of the monofilm technique of Langmuir and Blodgett get direct evidence that the physiological properties of a cell surface depend upon a particular molecular orientation.

*Effect of protein or fat diet on glucose oxidation.* W. H. CHAMBERS and J. P. CHANDLER (by invitation). Departments of Physiology and Biochemistry, Cornell University Medical College, New York.

Many investigators have reported a decreased tolerance for glucose following a diet low in carbohydrate. Our experiments demonstrate the effect on glucose oxidation of diets consisting solely of lean meat or of fat.

Four dogs were kept at a constant body weight for 2 to 3 weeks by feeding 600 to 900 grams daily of lean beef heart. In each case the basal R.Q. was 0.77, and after the administration of 50 grams of glucose the quotients averaged 0.92 to 0.94. This response is almost as great as that obtained after a preliminary diet of carbohydrate. Tolerance tests showed a hyperglycemia which was slightly greater and prolonged for about 2 hours beyond the normal.

An exclusively fat diet consisting of 125 grams daily of lard or butter was fed to three dogs for 3 or 4 weeks. The basal quotients averaged 0.73, and after the test meal of 50 grams of glucose the average R.Q. was 0.75 for 4 hours. An average of 11 grams of glucose was excreted. This reaction is quite similar to that obtained by a complete fast for the same length of time.

The meat contained about 0.1 per cent of carbohydrate, but 75 to 100 grams daily of potential carbohydrate may have been derived from it. Thus the ability to oxidize carbohydrate was maintained at an almost normal level. The 13 grams daily of potential carbohydrate from the glycerol fraction of the fat was ineffective in this respect.

*Insulin reactions in fasted dogs.* J. P. CHANDLER (by invitation) and WILLIAM H. CHAMBERS. Departments of Physiology and Biochemistry, Cornell University Medical College, New York.

The hypoglycemic response to insulin was observed in ten experiments on seven dogs in a well-fed condition, then again after a fast of 3 weeks' duration. After an intravenous injection of 0.25 unit of insulin per kgm. of body weight the blood sugar was determined at 15 minute intervals. The initial blood sugar level was lower in the fasted (average 60 mgm. per cent) than in the fed animals (average 76 mgm. per cent), but the general

form of the blood sugar curve was the same in both groups. The curves reached a minimum in the range of 30 to 40 mgm. per cent at about 30 minutes and then returned to the initial level in about 2 hours. Therefore the absolute fall in blood sugar in the fed condition averaged 37 mgm. per cent, as compared to 24 mgm. per cent in the fasted state, but if the fall in blood sugar is calculated as per cent of the initial level, there is little difference between the two conditions. A diet of fat produced the same effect as a fast.

In addition to the hypoglycemic reaction to insulin, which lasted not more than 2 hours, another effect on carbohydrate metabolism, of longer duration, was demonstrated in the same fasted dogs. We have shown previously that, when glucose alone is ingested after fasting, there is no appreciable rise in R.Q. in the first 4 hours, whereas, if insulin is administered with the glucose, the R.Q. rises to 0.80 from the basal level of 0.72. In the present experiments the basal R.Q. remained at the usual low level after insulin treatment. When glucose was given 24 or 48 hours later, the reaction was the same as when glucose and insulin were administered simultaneously, thus demonstrating a delayed effect of insulin on glucose oxidation. This effect was no longer apparent 4 to 6 days after injection. Observations of the hyperglycemia and glycosuria following the glucose meal were consistent with the respiratory data.

*Pancreatectomy in the monkey.* SARAH W. CHAPMAN (by invitation) and J. F. FULTON. Laboratory of Physiology, Yale University School of Medicine, New Haven.

Of 16 successfully depancreatized monkeys (*Macaca mulatta*), gross examination and histological section of suspected tissue at autopsy confirmed removal, complete in 12 animals and incomplete in 4. Six of those completely depancreatized died within 7 days but six others survived on a high carbohydrate diet *without insulin*, for 30–305 days. The chief cause of death were gastrointestinal disturbances attributable to the operation, extraneous infection, e.g., tuberculosis, or sacrifice. No animal died in acidosis or coma. On immediate postoperative fasting, glycosuria was present (5–8 grams/day) gradually diminishing to zero in the third to fifth day of fast. On feeding, glycosuria returned and could be increased to 85 grams/kilo/day by feeding *ad lib.* With feeding or fasting, ketonuria was intense for the first 8–14 postoperative days, disappearing thereafter and not reappearing even on fasting. On later fasts, glycosuria was absent by the second day and the animals readily went into hypoglycemia, the symptoms of which were relieved by sugar without insulin. Blood sugars fluctuated widely (14–410 mgm. per cent). The fasting nitrogen excretion was increased and the D/N ratios were not constant. Loss of weight was considerable (about one third of body weight) with muscular atrophy and absence of fat stores at autopsy. Total liver lipid was 19–23 per cent in the group dying within 6 days but within the normal range (2.5–7 per cent) in the long term animals. Crude saline extracts of anterior pituitary had little effect on those totally depancreatized, although they produced marked glycosuria and ketonuria in the partially depancreatized animals. Theelin treatment (1000 I.U. daily) in 3 monkeys did not diminish glycosuria. Subsequent hypophysectomy of one monkey caused reduced glycosuria and glycemia. It is concluded that in spite of obvious disturbance of carbohydrate metabolism, depancreatized monkeys, unlike depancreatized carnivora, do not die in typical diabetic acidosis.

*Photosensitive pigments from the frog's retina.* AURIN M. CHASE (introduced by Selig Hecht). Laboratory of Biophysics, Columbia University, New York.

Extracts of the drog's retina show the presence, not only of visual purple, but of another light-sensitive substance or substances. This is present in very low concentration compared with that of the visual purple; it is extremely sensitive to light, and its absorption spectrum is different from that of visual purple, with maximum absorption further toward the red. These characteristics make it probable that this pigment comes from the cones.

The demonstration of this pigment depends on the fact that it absorbs light of longer wavelengths than does visual purple. It is consequently possible to destroy its color by illuminating with wavelengths above 680 mμ without affecting the visual purple in the extraction. The form of the absorption spectrum, as well as the behavior of the solution during illumination, suggest the possibility of more than one substance.

*The occurrence of Thevetin and Kokilphin in the nuts of Thevetia ycottli.* K. K. CHEN and A. LING CHEN (by invitation). Lilly Research Laboratories and Indiana University School of Medicine, Indianapolis. (Read by title.)

From the nuts of *Thevetia ycottli* collected from Mexico, we have succeeded in isolating two crystalline substances; one of which proves to be identical with thevetin both chemically and pharmacologically, and the other identical with kokilphin. It is thevetin that is solely responsible for the digitalis-like action of these nuts. The name thevetosin originally proposed by Herrera can therefore be dismissed from literature.

*Toxicity determination in small animals by intravenous injection.* K. K. CHEN and CHARLES L. ROSE (by invitation). Lilly Research Laboratories and Indiana University School of Medicine, Indianapolis. (Demonstration.)

With small quantities of active material, it is often desirable to determine the toxicity in small warm-blooded animals. Holders suitable for intravenous injection in mice, rats, and guinea pigs will be demonstrated, and protocol forms will be exhibited.

*Effects of acetoacetate injections on blood sugar and liver glycogen levels.* JANE L. CHIDSEY (by invitation) and J. A. DYE. Department of Physiology, Cornell University, Ithaca, N. Y.

Intravenous injections of sodium acetoacetate were given to amytalized nephrectomized normal and hypophysectomized cats and dogs. Fifteen minutes after the injections the total blood acetone bodies varied from 71 to 282 mgm. per cent. This caused an initial hyperglycemia in 9 out of 11 cats, an effect which was absent in our dogs. These early blood sugar levels declined sharply in every animal receiving acetoacetate, but not in normal controls, reaching hypoglycemic proportions in 6 of 11 cats and 6 of 7 dogs.

From liver glycogen determinations it was demonstrated that acetoacetate injections led, without exception, to drastic hepatic glycogenolysis. Initial glycogen values varied from 0.58 to 6.27 per cent, final values from

0.049 to 3.41 per cent. Complete denervation of the liver and adrenals together with removal of one and demedullation of the other adrenal failed in 2 cats to check appreciably this glycogenolysis.

Marked hypoglycemia invariably occurred in normal animals injected with acetoacetate when the initial liver glycogen was low and in hypophysectomized animals regularly, under identical treatment, unless a high hepatic glycogen was assured by previous administration of carbohydrates. Some of these animals reacted like normal ones with high liver glycogen. Minimal blood sugar levels usually occurred between 1 and 2 hours after the injection. Levels as low as 15 mgm. per cent were observed, some terminating fatally. Glucose injections not only raised the blood sugar, but prolonged the life of the animal. Two such injections were required in 3 experiments, each being followed by a decline to hypoglycemic levels.

In experiments where marked hypoglycemic levels were not reached, partial recovery of blood sugar frequently occurred and was maintained to the sixth hour. This recovery usually coincided with a blood acetone body value below 45 mgm. per cent. In all cases in which the blood sugar failed to show a recovery after 2 hours, the initial liver glycogen was below 2 per cent and the final value below 0.2 per cent. Muscle glycogen did not vary significantly during the experiments.

*Influence of insulin, glucose, and alkalizing salts on the rate of disappearance of ethyl alcohol from the blood.* B. B. Clark and R. W. Morrissey (introduced by H. E. Himwich). Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y.

The oxidation of ethyl alcohol proceeds at a constant rate. It has been practically impossible to alter it by therapeutic measures. The value of insulin, glucose and sodium bicarbonate in the treatment of experimental methyl alcohol poisoning was demonstrated by Clark and Gibson (1933). It has been shown in this laboratory that insulin, glucose, and sodium bicarbonate significantly increase the rate of disappearance of ethyl alcohol from the blood. This report concerns the mechanism of this action.

Dogs were given ethyl alcohol in doses of 3 grams per kilogram. A control curve was obtained for each animal over a 6–8 hour period. For each individual experiment the control curve was established by hourly blood alcohol determinations for 3–4 hours. The therapeutic agent was then given and the blood alcohol followed hourly for the next 3–5 hours.

Neither glucose, sodium bicarbonate nor sodium citrate increased the rate of disappearance of ethyl alcohol from the blood. However, insulin (1–2 units per kilogram) produced in every experiment a definite increase in the rate, ranging from 20–50 mgm. per cent in 2–4 hours. Insulin plus glucose also accelerated the rate on the average by 30 mgm. per cent. The combined use of insulin, glucose, and an alkalizing salt such as sodium bicarbonate or sodium citrate produced the greatest decrease in blood alcohol, averaging 38 mgm. per cent. These animals were in better condition during the experiment and appeared to recover completely sooner than those in the other groups.

Insulin does not appear to increase directly the oxidation of ethyl alcohol, since its addition to liver tissue in the Warburg apparatus is without effect. Hypoglycemia probably does not force the tissues to burn more

alcohol as a source of energy, for usually when the blood sugar approached hypoglycemic levels the rate of disappearance of alcohol decreased; furthermore, acceleration was more often noted before hypoglycemia was reached. It seems probable, therefore, that the acceleration in the oxidation of carbohydrate by insulin catalyzes the oxidation of alcohol, thus increasing its rate of disappearance from the blood.

*Behavior of lymphatic capillaries as observed microscopically in the living animal.* E. R. CLARK and ELEANOR L. CLARK (by invitation). University of Pennsylvania, Philadelphia.

Our studies have provided evidence for the following conclusions: (1) that new growth of lymphatic capillaries has no apparent relation to the "need" for lymphatics, but occurs when mechanical and chemical conditions provide a growth promoting environment; (2) that the presence of a rich supply of lymphatics does not necessarily imply a correspondingly large amount of lymph flow; (3) that lymphatics, especially when protected from massage, may harbor debris, such as partially degenerated erythrocytes or leucocytes, for days, weeks, or months—shielding them from the action of phagocytic macrophages and of chemical substances in the intervascular spaces; (4) that some of the fluid and some of the erythrocytes and leucocytes found in the lymphatics pass into them from the blood vessels so directly that they cannot be considered as having played any part in the metabolic activity of the tissue cells, but should be considered as constituting a "leakage" from blood vessel to lymphatic, a "leakage" which increases with changes affecting the consistency of endothelium and which may, following mechanical or chemical insults, reach the stage of direct hemorrhage into the lymphatic; (the relative amount of this "leakage" under various conditions is unknown, but its occurrence casts very strong doubt on the validity of the interpretation of the content of the lymphatic as identical with tissue "fluid";) (5) that these and other data suggest the necessity for a revision of some of the time-honored views regarding the function of the lymphatic system.

*The permeability of the lens capsule to calcium.* JANET HOWELL CLARK. School of Hygiene and Public Health, Johns Hopkins University.

Lens protein, extracted in 0.5 per cent potassium chloride at pH 7.2, showed no increase in opalescence after exposure to ultraviolet radiation and heating to a moderate temperature but extracts in Ringer solution, containing varying amounts of calcium chloride, developed an opalescence which increased with the amount of calcium present. This made it possible to develop a method of studying the permeability of the lens capsule to calcium.

Lenses were removed without injuring the capsule and soaked for an hour in Ringer solution containing 0.1 per cent calcium chloride to which various substances were added which might be expected to affect the permeability of the lens capsule. The lenses were then removed from the solution and extracted in 0.5 per cent KCl. The extracts were radiated, heated to a moderate temperature, and the degree of opalescence determined by means of a Tyndall-meter. The degree of opalescence was a measure of the amount of calcium in the lens. Lenses from freshly extracted eyes were practically impermeable to calcium but the permeability increased if the eyes were kept on ice for a day or more.

Exposure to ultraviolet radiation during immersion in the solution containing calcium chloride did not affect the permeability of the capsule to calcium, nor was it affected by slight changes in hydrogen ion concentration or temperature. Addition of levulose, dextrose or viosterol to the solution did not affect the permeability of the capsule to calcium but addition of parathyroid extract produced a marked decrease in the permeability to calcium. The number of units of extract necessary to prevent the penetration of calcium into the lens was proportional to the concentration of calcium salts in the solution.

*The time relations of chest leads.* PAUL CLARK (by invitation) and JANE SANDS ROBB. Student Health Service and Department of Pharmacology, Syracuse University.

The Committee of the American Heart Association on the Standardization of Precordial Leads has recommended that the standard nomenclature P, Q, R, S, and T be used for the new Leads 4 B, R, L, F, and T respectively. With our present facilities for recording six leads simultaneously we have obtained data which supports the ruling of the committee. It should be noted, however, that the corresponding waves in the three standard leads are seldom synchronous and this is more conspicuous in Chest Leads. Differences of 5 to 15 Sigma are commonly obtained. These differences are characteristic neither of the wave nor the individual. The phase-difference may arise from stature, respirations, posture, opening the abdomen, and undoubtedly from various pathological states. In normal monkeys and in normal medical students the following sequences of R-waves were frequently observed: Lead 4 F preceded 2; 4F, 1, 2, 3; 1, 2, 4F, 3; 2, 3, 1, 4F; 1, 2, 3, 4F.

Acknowledgment is made to the Hendricks Fund for support.

(Slides)

*The sexual skin of the baboon.* ROBERT W. CLARKE. Laboratory of Physiology, Yale University School of Medicine, New Haven.

Krohn and Zuckerman (J. Physiol. **88:** 369, 1937) described the weight changes of a pig-tailed macaque during the menstrual cycle, attributing them to swelling and subsidence of the sexual skin. Observations on a mature, non-pregnant hamadryas baboon showed similar changes in bulk of the sexual skin. Though disturbed by anesthesia, her menses recurred every 32 days and were characterized by a huge swelling which started after menstruation and abruptly receded about the 14th day. The volume of the sexual skin was measured by immersing the dependent organ in a large vessel of water poised on a springless balance, and noting the rise in weight. Complete immersion of the sexual skin exactly to its junction with the animal's rump is readily effected and the results appear accurate to 0.2 liter. Extracellular fluid was measured by the thiocyanate method (Lavietes, et al. J. Clin. Invest. **15:** 261, 1936) under nembutal anesthesia. Condensed tabular data show the sequence of weight and volume changes. Anesthesia or thiocyanate on day 17 were possibly responsible for prolongation of the period to 41 days. Specific gravity of the sexual skin was taken as 1.05. The increased volume of the sexual skin results not only from retention of water but also from considerable shift of body water

| DAY OF MENSTRUAL CYCLE | TOTAL WEIGHT | SEXUAL SKIN VOLUME | INCREASE OVER RESTING STATE | | WEIGHT SHIFT: BODY TO SEXUAL SKIN | EXTRACELLULAR WATER | |
|---|---|---|---|---|---|---|---|
| | | | Total weight | Sexual skin | | Volume | Per cent of total weight |
| | *kgm.* | *l.* | *kgm.* | *kgm.* | *kgm.* | *l.* | *per cent* |
| −5 | 10.4 | 0.5 | 0 | 0 | 0 | 2.8 | 27 |
| 3 | 10.6 | 0.9 | 0.2 | 0.4 | 0.2 | | |
| 5 | 11.1 | 1.4 | 0.7 | 1.0 | 0.3 | | |
| 9 | 10.8 | 1.8 | 0.4 | 1.4 | 1.0 | | |
| 17 | 11.1 | 1.8 | 0.7 | 1.4 | 0.7 | 3.3 | 30 |
| 25 | 12.1 | 2.6 | 1.7 | 2.2 | 0.5 | | |
| 27 | 12.2 | 3.1 | 1.8 | 2.8 | 1.0 | | |
| 32 | 13.0 | 3.9 | 2.6 | 3.6 | 1.0 | | |
| 35 | 12.5 | 3.6 | 2.1 | 3.3 | 1.2 | | |
| 37 | 11.3 | 2.1 | 0.9 | 1.7 | 0.8 | | |
| 38 | 10.8 | 1.4 | 0.4 | 0.9 | 0.5 | | |
| 41 | 10.4 | 0.8 | 0 | 0.3 | 0.3 | | |

*Blood pressure studies in adrenalectomized dogs.* R. A. CLEGHORN, C. W. J. ARMSTRONG (by invitation) and D. C. AUSTEN (by invitation). Department of Medicine, University of Toronto.

The vascular paralysis described by Elliott in 1914 as occurring in cats moribund from adrenal insufficiency has also been found in adrenalectomized dogs just prior to death. In these animals neither splanchnic nerve stimulation nor the injection of nicotine, pitressin or barium chloride caused much rise in blood pressure as a rule. Epinephrine, however, still elicited a substantial rise in pressure. While such were the findings in many dogs examined after the onset of the terminal weakness, others at a stage clinically indistinguishable from these showed pressor responses to the various procedures which, though less than normal, were quite marked. This may be explicable, at least in part, on the basis of the development of a terminal bradycardia in which heart rates of 30 to 40 per minute were commonly observed, failure in cardiac function occurring prior to exhaustion of the peripheral vascular system.

That the loss of the adrenal medulla is not responsible for the impaired capacity for contraction to pressor procedures in adrenal insufficiency is indicated by the fact that adrenalectomized dogs maintained many months by injections of adrenal cortical extract show responses which are substantially normal. The unresponsiveness in adrenal insufficiency is probably due to electrolyte and other changes occurring during the development of that condition rather than to the absence of the cortical hormone, for adrenalectomized dogs maintained in health by a high salt, low potassium diet exhibit excellent increases in blood pressure to splanchnic nerve stimulation to nicotine and barium chloride.

*The blood histamine in anaphylactic shock.* C. F. CODE (introduced by F. C. Mann). Division of Experimental Medicine, The Mayo Foundation, Rochester, Minnesota.

In this study the blood histamine was estimated by a modification of the Barsom-Gaddum method. Guinea pigs were sensitized by the subcutaneous injection of diluted egg white. Following a suitable interval blood was taken just before and after introduction of the antigen into the

blood stream. The blood histamine of those animals showing unmistakable signs of bronchial spasm was increased from 3 to 10 times the control value. Blood withdrawn under similar conditions from normal guinea pigs showed no significant alteration in its histamine equivalent.

A more detailed investigation was made of the blood histamine in dogs during anaphylactic shock. These animals were rendered sensitive to horse serum, egg white and duck serum. Injection of doses which prior to sensitization caused no change in the blood histamine or general health of the animal produced after sensitization a sharp rise in the blood histamine associated with the classical signs of anaphylactic shock in this species. The blood histamine generally reached its maximum within six minutes of the injection of the antigen, then fell rapidly toward its former level. The sudden increase in histamine was coincident with the dramatic fall in the blood pressure. During anaphylactic shock not only was the total quantity of active substance increased in the blood, but its distribution was altered. In the control unclotted blood the plasma was histamine free and the white cell layer was the source of most of the activity, but during anaphylactic shock the plasma contained the active substance and at the peak of the reaction was its major source.

*Electric impedance of Nitella during excitation.* KENNETH S. COLE and HOWARD J. CURTIS (by invitation). College of Physicians and Surgeons, Columbia University.

The membrane impedance characteristics of the long single cells of the fresh water plant *Nitella* are practically identical with those of single nerve fibers (H. J. Curtis and Cole, K. S., J. Gen. Physiol. **21:** 189, 1937; Curtis, H. J. and Cole, K. S., in press), and their propagated waves of excitation are similar except for velocity. The changes in the alternating current impedance during this excitation wave in *Nitella* have been measured with the current flow normal to the cell axis and at 7 frequencies from 0.10 to 10 kilocycles per second, with simultaneous records of the action potential under the impedance electrodes. At each frequency the resting cell was balanced in a Wheatstone bridge with a cathode ray oscillograph, and the changes in the complex impedance during activity were determined from the bridge unbalance recorded by motion pictures of the oscillograph figure. An extension of the previous technique of interpretation of the transverse impedance shows that the normal membrane capacity of $0.9\mu f/cm^2$, decreases about 15 per cent without change of phase angle while the membrane resistance decreases from probably $2.10^5$ ohm $cm^2$ to about $10^3$ ohm $cm^2$ during the excitation wave.

*Localization and physiologic significance of the aortic chemoreceptors in the dog.* J. H. COMROE, JR. (introduced by Carl F. Schmidt). Laboratory of Pharmacology, University of Pennsylvania, Philadelphia.

In 49 dogs under morphine chloralose anesthesia, bilateral carotid denervation usually reduced but never abolished hyperpnea produced by $N_2O$, NaCN, and $\alpha$ Lobelin. Carotid reflexes rarely contribute to, but usually mask hypertension produced by these agents for their denervation invariably accentuates hypertension following intravenous NaCN, $\alpha$ Lobelin, usually intensifies that following $N_2O$. Hypertension is completely abolished by subsequent vagotomy (except for ganglionic effect of $\alpha$ Lobelin in large dosage). These agents raise blood-pressure

almost entirely through vagodepressor reflexes for, with few exceptions, they fail to produce significant hypertension if vagi are blocked, carotid innervation intact. Stimulation of aortic chemoreceptors alone by these agents produces moderate hyperpnea, marked hypertension, no immediate bradycardia; stimulation of carotid chemoreceptors alone produces intense hyperpnea, never significant hypertension, often immediate bradycardia (if last factor is eliminated by vagotomy, carotid chemoreceptors occasionally produce slight, rarely produce marked, hypertension).

Localization of aortic chemoreceptors was accomplished by injections of $\alpha$ Lobelin and NaCN via catheters passed through aorta, carotid and subclavian arteries after denervating carotids; sensitive region was consistently found in first portion of aortic arch, on cardiac side of brachiocephalic orifice. Intraaortic injections at this point regularly produced marked, immediate hyperpnea and hypertension which vagotomy abolished. At this point a small vessel regularly leaves aorta to supply Aortic Body (Paraganglion Aorticum Supracardiale) which lies in aortic adventitia less than a millimeter away. Afferent nerves from aortic body enter vagus (on right side certainly, on left side probably) with recurrent laryngeal; electrical stimulation of aortic body nerve to right vagus produced hyperpnea and hypertension similar to that following intraaortic NaCN or $\alpha$ Lobelin. MacDowall reflex may depend largely on stimulation of aortic body receptors by anoxemia. In cyanotic dogs with carotids denervated vagotomy or $O_2$ inhalation often causes abrupt fall in blood-pressure, receptors being thus inactivated.

Conclusions: Stimulant effects of $N_2O$, NaCN, $\alpha$ Lobelin *on respiration* are due to reflexes from carotid and (to a lesser degree) aortic bodies, those *on circulation* almost entirely to reflexes from aortic body, arterial and nerve supply of which are described.

*The possible rôle of the sinus caroticus in respiration in the cat.* Helen C. Coombs and Otis M. Cope. Department of Physiology and Biochemistry, New York Medical College. (Read by title.)

Investigation has been made of the rôle which might be played by the sinus caroticus in respiration under ordinary conditions in cats. Respiration was recorded by stethographs placed around the upper part of the chest and at the level of the diaphragm and connected to tambours. Anesthesia was maintained by ether or nembutal. The results were:

I. *With afferent nerves intact.* The sinus caroticus was (a) excited with a mild induced current, (b) frozen with ethyl chloride and (c) deprived of oxygenated blood by serraffins above and below the sinus. A small increase in depth (not rate) of *costal* respiration appeared. This was also elicited by excitation of the ninth nerve. The effect was somewhat more apparent under nembutal than under ether. (15 cats)

II. *With part of the afferent pathway interrupted.* This was done by (a) section of the dorsal spinal roots in the thoracic region, (b) division of both vagi and (c) section behind the posterior colliculi. The results were:

(a) Following section of the dorsal roots, with diminished costal respiration, excitation of the sinus was ineffective in altering depth or rate of costal respiration. (b) After division of both vagi, excitation of the sinus at times caused a slight increase in the depth of costal respiration. The effect was neither consistent nor unequivocal. (c) After section

behind the posterior corpora quadrigemina, excitation of both sinuses produced no additional effects. (30 cats)

III. *With diaphragmatic respiration abolished by phrenic avulsion* costal respiration was, of course, greatly increased. Excitation of the carotid sinus was without effect. Freezing the sinus temporarily reduced it. The same effects followed excitation or freezing of a portion of the ninth nerve. (5 cats)

The sinus caroticus would appear to have no greater importance in respiration in mammals than other endings of the ninth nerve. This is less than its importance in fish. (Springer, M. G., Arch. Neurology and Psychiatry, 1928, xix, 834.)

*Observations on the relation of experimental hypertension to renal clearance and renal ischemia.* A. C. Corcoran (by invitation) and Irvine H. Page. Lilly Laboratory for Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana.

Observations of the changes of renal urea and creatinine plasma clearances which occur in uni-nephrectomized dogs after constriction of the renal artery (Goldblatt) have shown that it is possible to excite a persistent arterial hypertension without altering the clearances.

Similar observations were made with reference to plasma urea, inulin, and phenol red clearances. The relative and absolute values of these clearances also are not necessarily altered by constriction of the renal artery, although a temporary decrease of phenol red clearance usually occurred during the twenty-four hours after operation.

The significance of these results is discussed with reference to the concept of renal ischemia as the exciting cause of experimental renal hypertension.

*Further observations on carbohydrate metabolism in hypophysectomized rats following cortico-adrenal extract administration.* E. L. Corey. Physiological Laboratory, University of Virginia.

There is abundant evidence that both the hypophysis and adrenal cortex are intimately involved in carbohydrate economy. It has been suggested that the hypophysis may control sugar formation from protein through the adrenal, and that cortico-adrenal atrophy might be a major factor in bringing about carbohydrate reduction following hypophysectomy. Recent reports indicating that injections of adrenocorticotropic hormone may maintain the tissue glycogen of hypophysectomized rats has given support to this view.

If this hypothesis be correct, it should follow that the administration of cortico-adrenal extract to hypophysectomized animals should prevent, to a considerable degree, the lowering of carbohydrate levels which would otherwise occur.

To test this hypothesis about 100 immature and adult rats were hypophysectomized and fasted for periods of 12, 24 and 48 hours. During this interval they received injections (6 to 20 cc.) of cortico-adrenal extract (53 animals), adrenalin (7), NaCl (10), or were left untreated (17). Twenty unoperated animals, similarly fasted, received no treatment (12) or injections (20 cc.) of extract (8).

All of the hypophysectomized rats treated with extract showed blood

sugar levels equal to, or in excess of (av. 90 mgm. per cent) those seen in unoperated controls. Bioassays of these extracts by the blood-pressure method failed to reveal the presence of adrenalin.

On the other hand, all of the experimental animals showed markedly reduced liver and muscle glycogen values (av. 0.15 mgm. per cent) whether injected with extract alone, extract plus adrenalin, extract plus glucose, or "primed" with extract for several days before fasting.

It appears from the present experiments that the liver and muscle glycogen losses which follow pituitary ablation cannot be considered as mainly attributable to cortico-adrenal atrophy. Nevertheless, the high blood sugar levels observed in all of the extract-injected animals may be highly significant.

*Production of ketone bodies and glucose by the liver in unanesthetized dogs during fasting and fat feeding.* LATHAN A. CRANDALL, JR. and G. J. EHNI (by invitation). Northwestern University Medical School, Chicago.

Determinations of glucose and total ketone bodies in the portal venous, arterial, and hepatic venous blood have been made in unanesthetized dogs angiostomized by the London technique. The animals were subjected first to several days of fasting, following which olive oil, peanut oil, or lard was given by mouth in amounts adequate to supply the total caloric requirement. In dogs fasting eighteen hours there is no detectable formation of ketone bodies by the liver; the glucose output from the liver is comparable to that previously reported, averaging about 10 mgm. per 100 cc. of blood. During 3 to 7 days of fasting the blood ketone bodies rise, and concentration of ketone bodies in the hepatic venous blood substantially exceeds that in the blood reaching the liver. Following the feeding of large amounts of fat to the previously fasted animals there is a further increase in the rate of ketone body production by the liver and the general blood level tends to rise further. There is a tendency for the hepatic output of glucose to decrease during fasting and fat feeding, and for the blood sugar level to drop below the 18 hour fasting value.

Estimates of total ketone body production based on the values that have been given for hepatic blood flow and on our figures for ketone formation per 100 cc. of blood indicate that the oxidation of these substances could furnish an appreciable fraction of the total caloric requirement.

Arterio-portal ketone body differences show that the gastro-intestinal tract removes ketones from the blood in appreciable amounts.

*A further study of the inhibitory effect on gastric peristalsis of the products of protein digestion.* J. O. CRIDER and J. EARL THOMAS. Jefferson Medical College, Philadelphia.

In 1936 we reported that certain commercial peptones cause inhibition of gastric peristalsis when placed in the upper small intestine.

To learn whether the products of protein digestion in the living animal have a similar action, the proteose fraction was separated by ammonium sulphate precipitation from intestinal contents collected from dogs while they were digesting lean meat. After removal of fat and reagents, the material was prepared in isotonic, neutral solution containing about 5 per cent of proteose. Ten or 20 cc. of this solution, when placed in the

upper small intestine of a dog, caused gastric inhibition comparable to that caused by similar amounts of the moderately active commercial peptones.

The effect of peptone as distinguished from proteose was studied, using proteose-free fractions of a commercial peptone (Neopeptone, Difco) separated by means of differences in alcohol solubility. The various fractions caused different degrees of gastric inhibition but some of them were fully as active as proteose. Similar results were obtained with the proteose-free residue of the intestinal contents.

The inhibitory effect on gastric peristalsis of the total intestinal contents, exclusive of HCl, is about half due to proteose and half to other substances including peptone. Acidity was found to be a minor factor. Comparing untreated intestinal contents with the same material after neutralization no difference in inhibitory action could be demonstrated unless the untreated material was more acid than pH 3.0. The same is true of acidified commercial peptone solutions.

Experiments were performed on two vagotomized dogs. Witte's Peptone was used in most of the trials because it has the greatest inhibitory effect of any of the preparations so far studied. Except for a few results of doubtful significance, the peptones used had no effect on gastric peristalsis after double vagotomy. We conclude that the inhibitory effect is the result of a reflex dependent on the vagi and is, therefore, a manifestation of the enterogastric reflex.

*The distribution of water and chlorides following massive infusions of isotonic glucose solution.* R. A. Cutting, A. M. Lands and P. S. Larson (introduced by T. Koppanyi). Department of Physiology, Georgetown University School of Medicine, Washington, D. C.

When isotonic solutions of chemically pure glucose (5 per cent) are infused into cats through the femoral or external jugular vein at rates approximating 4 cc. per kgm. of body weight per minute (maximum tolerated rate) death occurs when 368–535 cc. of fluid per kgm. body weight have been administered.

The range in magnitude of the "lethal dose" is due to variations in the rate of elimination of fluid by the animals' excretory channels during the infusion. The lethal value for "retained fluid" is approximately 292 cc. per kgm. body weight (maximum variation 9 per cent).

Changes occur in the arterial blood pressure during massive infusions. In general the mean arterial pressure (normally 110–170 mm. Hg) undergoes a progressive increase of 10–30 mm. during the first half hour, after which it declines gradually to a value of 40–80 mm. and then in the 10 minute period immediately preceding death declines precipitately. The terminal decline is associated with bradycardia.

No entirely satisfactory explanation of the pattern of distribution of the retained water has yet been found. The spleen and brain, for example, imbibe little or no water, whereas the stomach and pancreas store considerable (increasing their water content by 112 per cent and 342 per cent respectively) and other tissues, muscles, skin, kidney, liver, salivary glands, etc., imbibe water in intermediate degree. Muscle, blood and skin constitute the most important reservoirs for water storage because of their relative mass in the body. Muscular tissue accounts for approximately 23 per cent, blood 17 per cent, and skin 10 per cent (total 50 per cent) of the retained water.

During infusions small amounts of chlorides are excreted in the urine; calculated as sodium chloride the loss averages 0.092 gram per kgm. body weight. Chlorides are excreted principally during the initial stages of the infusion; terminally they are present only in traces.

As a result of infusion chlorides undergo redistribution amongst the various tissues. Allowing for tissue dilution some organs show relative gains of chloride (submaxillary gland 10 per cent, pancreas 148 per cent). Blood shows neither gain nor loss. Other tissues show relative losses varying from 1 per cent in the case of the large intestine to 60 per cent in the case of the liver.

*Treatment of acute barbiturate poisoning by "lavage du sang."* R. A. CUTTING (by invitation) and T. KOPPANYI. Departments of Physiology and Pharmacology, Georgetown University School of Medicine, Washington, D. C. (Motion picture.)

The following is a motion picture demonstration showing recovery of cats acutely poisoned with barbiturates and treated by "lavage du sang." The latter term is borrowed from Dastre and Loye (1888). They used it to designate the cleansing effect produced on blood by the short sojourn in the circulation of physiological salt solution introduced in quantity by vein. It has been repeatedly observed by one of the authors (R. A. C.) that cats (or dogs) given anesthetic doses of barbiturates and subsequently used for experiments involving the administration of massive infusions of isotonic solutions of sodium chloride or glucose show remarkably short recovery times. Employing doses of 600–800 cc. per kgm. body weight of 1 per cent sodium chloride solution or from 300–400 cc. per kgm. of 5 per cent dextrose solution (injection rate 7–10 cc. per min.) we have shown,

1. That in cats receiving 100 mgm. sodium phenobarbital or 200–300 mgm. sodium barbital per kgm. body weight recovery time is shortened to one-third to one-half that of controls and
2. That the early elimination of barbiturates in the urine is more than double that of controls.

By prolonging massive infusions over a period of four or five hours it has been possible to cause the recovery, within 18 hours, of cats receiving doses of 600–1000 mgm. of sodium barbital (i.e., twice or thrice the fatal dose) per kgm. body weight. Notwithstanding the fact that massive infusions produce diuresis and this diuresis causes increased elimination of the barbiturates in the urine, the elimination of barbiturates by the kidney is not solely responsible for the speedy recovery. Animals receiving sodium barbital in doses of 600 to 1000 mgm. per kgm. were found to be fully recovered at a time when only about one-third of the total amount had been excreted in the urine.

This means that except for destruction by metabolic processes two-thirds of the dose still remained in the body.

*The effect of sulphydryl compounds on milk production.* RAY G. DAGGS. University of Vermont.

The author and co-workers have shown previously that proteins or protein degradation products that contain sulphur act as mammary gland stimulants.

Lactation studies were carried out on rats. The sulphydryl amino

acids existing either as such, (cysteine) or in the potential form, (cystine, methionine) are dietary lactagogues. The non-sulphur amino acids of the tripeptide glutathione, glycine and glutamic acid, have no lactogenic action. In the intermediary metabolism of the sulphydryl compounds, the lactogenic effect is not entirely destroyed at the taurine stage. The essential growth principle, lysine, is not a factor in the problem of dietary lactagogues. The catabolic pathway of taurine to form taurocholic acid in the process of milk production is improbable. It is not possible to surpass the animal's hereditary capacitance for lactation by increasing the sulphur amino acid content of the diet to a higher level. There is no relationship between fatty infiltration of the liver and milk production.

Evidence is shown from four cases for the action of cystine as a human lactagogue. All cases were multiparas having a history of either failing or no milk supply with previous babies. Three cases involved observations made only during hospitalization. The fourth case was studied more intently.

*Daily gonadotropic hormone determinations during forty complete menstrual cycles.* FRED E. D'AMOUR, DOROTHY FUNK (by invitation) and HELEN LIVERMAN (by invitation). University of Denver, Denver, Colo.

The results of day by day tests for gonadotropic hormone in the urine of normal women during approximately 40 complete menstrual cycles will be reported.

These tests were made first to determine whether the results obtained on a relatively large number of cycles would be consistent with the accepted view that ovulation occurs during the mid-interval of the cycle; and second, whether the test could be sufficiently simplified, particularly as regards the volume of urine necessary, to have practical value as a diagnostic aid in certain cases of sterility.

The results to date (32 cycles) are as follows. Positive responses were obtained in the mid-interval (14th to 16th days preceding the next menstruation) in 24 of the 32 cycles, 5 others gave positive reactions at either side of the mid-interval and 3 were completely negative (anovulatory menstruation ?). But in 10 cases two equally positive reactions appeared during a single cycle, usually 10 days apart and usually during long cycles and in 9 cases positive responses were found within two days of menstruation. This may explain the frequent failures reported in the use of the "safe period" technic.

It was found that the night excretion of urine—a convenient amount for the subject to collect—is sufficient for test purposes, ovaries weighing 50 to 100 mgm. being produced in the immature rat. With proper precautions the rat tolerates this amount well and the method seems to offer a reasonably simple means of determining whether hypophyseal failure to stimulate ovulation is the underlying cause of certain cases of sterility.

*Absence of predilected sites for implantation of ova in the rabbit.* D. N. DANFORTH (by invitation) and A. C. IVY. Department of Physiology, Northwestern University Medical School, Chicago, Illinois. (Read by title.)

Certain anatomic considerations mentioned by Hartman (Sex and Internal Secretions, 1932) and evidence presented by Krehbiel (Anat. Rec. 51: 64, 1931) raise the question whether anatomical peculiarities

exist in the uterine mucosa for the predetermination of the implantation site in pluriparous and uniparous animals. Further, in pluriparous animals the ampullae, when present, act in labor as individual units, which prevents crowding in the corpus uteri and the vagina. This study was undertaken to determine whether such predetermined sites exist in the rabbit. If they do exist, one should expect the implantations to occur at the same sites in successive pregnancies.

Six rabbits were used in this study. They were mated, and when pregnancy had become well established they were operated under ether anesthesia. Black silk suture knots were placed on the serosal surface, one at the center of each placenta to serve as a marker. The abdomen was then closed, the animal allowed to recover from the operation and to deliver its young, and to mate again. On the 25th day of pregnancy the rabbits were killed and the relation of the new placentae to the previously placed markers was observed. In the first series of pregnancies there were in all 47 placental sites, all of which were marked at operation. In the second series there were 52 placentae. Of the 52 placental sites in this latter series, there were only 11 which corresponded closely to the previously placed sutures, or in which the border of the second placental site fell within the borders of the first placental site. The borders of 36 were from 1 to 2 cm. from either border of the marked sites, but none corresponded more closely than 1 cm. The borders of the remaining 5 placentae were more distant than 2 cm. from the first placental sites. From these results one may conclude that in the rabbit predetermined sites for implantation do not exist in the uteri.

*Contraction and evacuation of the gall bladder in the rabbit in response to cholecystokinin.* D. N. DANFORTH (by invitation), H. DOUBILET (by invitation) and A. C. IVY. Northwestern University Medical School, Chicago. (A motion picture in color.)

Practically complete contraction and evacuation of bile from the gall bladder of the albino rabbit was obtained in three of six rabbits by the single intravenous injection of two dog units of cholecystokinin. Seventy-five per cent evacuation was obtained in the others. The film shows in addition to a progressive decrease in the size of the gall bladder annular contractions like those described by Walsh (Am. J. Physiol. **100:** 594, 1932).

*Vitamin K assays.* FLEMINTINE PEIRCE DANN (introduced by A. C. Ivy). Abbott Laboratories, North Chicago, Ill.

These data on the biological assay of vitamin K were collected in conjunction with work started by Doctors Butt, Osterberg and Snell of the Mayo Clinic (1) to determine whether vitamin K is of importance to normal blood coagulation in the human and if so how it functions, (2) to investigate sources for vitamin K in nature and (3) to make vitamin K concentrates from these sources.

Dam and Schønheyder developed a curative method for the quantitative assay of vitamin K. The technique was difficult and time-consuming. Almquist and Stokstad described a preventive method for vitamin K which was rapid yet sufficiently accurate for comparative purposes and for a preliminary survey. Using this method our negative controls gave

a blood-clotting time of over thirty minutes in 46.8 per cent of the chicks, while the positive controls showed an average blood-clotting time of less than one minute. Macroscopic hemorrhages were observed in 10 to 20 per cent of the vitamin K deficient chicks at the end of a two weeks assay period.

Materials showing definite vitamin K potency by the Almquist technique are now being checked by the Dam and Schønheyder method. Perhaps a certain material assayed by the quantitative Dam and Schønheyder technique may later serve as a reference standard in a modification of the Almquist and Stokstad method.

*Results.* Dried human bile equivalent to 97.5 per cent of liquid bile in the diet gave no vitamin K protection, while dried human acholic feces at 2½ per cent of the diet was protective. The ether extracted fat from residual hog liver after aqueous extraction was effective when the diet contained 0.5 per cent, equivalent to 7.5 per cent whole liver. The non-sap fraction of soy bean oil equivalent to 7.7 per cent of the original oil in the diet was not protective, presumably due to the destruction of vitamin K by alkali. A vitamin K concentrate made from fish meal previously exposed to spontaneous bacterial decomposition and submitted by Doctors Butt, Osterberg and Snell was very potent and was as protective at a level of 0.001 per cent of the diet as 0.01 per cent of an iso-pentane extract of alfalfa.

*A study of simultaneous reactions of different autonomic indicators.* CHESTER W. DARROW and ERNST GELLHORN. Institute for Juvenile Research and Department of Physiology, College of Medicine, University of Illinois, Chicago.

Autonomic effects of excitation concern not only the immediate response to stimulation but also the course of events during the return of the organism to equilibrium. To study these processes composite records of simultaneous changes in narcotized cats have been obtained by a combined photographic and kymographic method. The following definite autonomic indicators have been used:

1. The sympathectomized pupil of one side, the dilatation of which indicates inhibition of the parasympathetic innervation.

2. The denervated nictating membrane (N. M.) of the same side, the contraction of which indicates discharge of adrenin.

3. The normally innervated N. M. of the opposite side, the contraction of which, in the absence of contraction of the denervated N. M. indicates sympathetic excitation.

Also recorded were: 4. Blood pressure; 5. The galvanic skin reflex, and 6. The normally innervated pupil.

Under reflex faradic stimulation (sciatic, femoral, brachial plexus, central end of vagus) dilatation of both sympathectomized and normal pupils has appeared even when the reaction of other autonomic indicators has been delayed or lacking. Absence or delay of pupillary response to stimuli affecting other autonomic indicators is shown if the state of the pupil before excitation is one of complete constriction. It is shown that even adrenin sufficient to occasion contraction of the N. M. may be inadequate to produce dilatation of such a contracted pupil. A minimal dose of atropin is shown to facilitate the response of such a pupil to reflex

stimulation and to adrenin. A constant 60 cycle stimulus to the peripheral end of the cut sympathetic nerve leading to the pupil has also been employed to maintain a minimal dilatation favorable to pupillary response.

Dilatation of the sympathectomized pupil may occur simultaneously with either a rise or a fall in blood pressure.

It is found that a condition of the narcotized animal disposing toward tetanic contractions or clonic movements in association with the faradic excitation favors a drop in blood pressure even after strong rapid stimuli. Curare in such animals may change the effect of strong rapid stimuli to an increase in blood pressure. Stretching of the mesentery is shown to favor responses attributable to inhibition of parasympathetic tone, whereas metrazol favors those indicating a sympathetic excitation.

Galvanic responses in animals with initially good reactions became minimal or absent as a state of surgical anesthesia (urethane, chloralose, chloralosane, ether, chloroform) was approached. The dependence of the galvanic response on cortical function is suggested.

Effects of oxygen deficiency on the magnitude and pattern of autonomic responses are demonstrated.

*An analysis of the factors involved in the changes in coronary blood flow in aortic stenosis and regurgitation.*[1] J. C. Davis (introduced by M. B. Visscher). Department of Physiology, University of Minnesota.

It was thought that some heretofore unknown factors may be involved in the alteration of the coronary blood flow in aortic stenosis and regurgitation. Experiments were therefore carried out using the heart lung preparation of the dog. Minute volume flow determinations through the circumflex coronary artery were made with a stromuhr. Arrangements were made so that one could alternately determine coronary blood flow per minute at pulsatile aortic pressures or at constant perfusion pressures. Aortic stenosis was produced by two methods. Aortic regurgitation was produced by means of instruments similar to those used by Wiggers.

When perfusion is taking place from the pressure bottle, any drop in diastolic pressure that occurs as a result of the valve lesion is not a factor per se in the coronary flow. The following data were obtained in a representative experiment:

*Coronary flow in cubic centimeters per minute*

| Perfusion directly from aorta | | Perfusion from pressure bottle | |
|---|---|---|---|
| Normal | 27.97 | Normal | 34.14 |
| Aortic stenosis | 24.39 | Moderate stenosis | 28.08 |
| Moderate regurgitation | 22.69 | Marked regurgitation | 11.25 |
| More marked regurgitation | 9.22 | | |

It can be seen that in both aortic stenosis and aortic regurgitation a definite drop in coronary flow occurs independent of changes in the aortic pressure. In order to determine what factors are involved in this decrease in coronary flow, the hot wire anemometer was used so that one could analyze changes taking place in a single cardiac cycle.

In both conditions a decrease in coronary flow was found both in systole and diastole due to elevation of intraventricular pressure during both periods.

[1] This work was supported by a grant (to Dr. Jay Conger Davis) from the Research Council of the American Medical Association.

*Reduction of experimental polycythemias by liver administration.* JOHN EMERSON DAVIS (introduced by A. D. Keller). Department of Physiology and Pharmacology, University of Alabama School of Medicine.

The basal erythrocyte numbers and hemoglobin percentages of four dogs were increased by the daily running of two and a half miles on a motor driven treadmill for a few weeks. In seven dogs, experimental polycythemia was induced by the daily oral administration of 2 mgm. of cobalt, as cobalt chloride, per kilogram of body weight. The increases in red cell numbers amounted to about 20 per cent in all dogs.

In spite of continued stimulation of the hematopoeitic tissue by exercise or cobalt, the daily feeding of 75 grams of whole hog or beef liver promptly reduced the erythrocyte numbers of all of the exercised dogs and six of the seven cobalt fed dogs to or below their normal values. The reticulocyte percentage, which was observed in three dogs, was increased 200 to 300 per cent by cobalt feeding and reduced by about the same amount when liver was fed in addition. The erythrocyte counts and reticulocyte percentages remained depressed throughout the periods of liver feeding (varying from 6 to 28 days) and arose to polycythemic levels promptly upon cessation of liver administration. Leukocyte counts remained fairly constant throughout.

The daily intramuscular injection of 0.5 cc. of liver extract into two polycythemic (exercise) dogs and into two polycythemic cobalt-fed dogs caused significant (12 to 20 per cent) decreases in the red cell counts which returned to polycythemic levels soon after discontinuation of liver extract injections.

The daily oral administration of 5 grams of stomach extract (Ventriculin) and of anti-anemic liver extract heated during preparation did *not* reduce significantly the high erythrocyte counts of three dogs which received daily treadmill exercise.

These results may be interpreted by the postulation of a liver hormone, quite distinct from anti-anemic principles, which has the function of depressing the hematopoeitic activity of the red bone marrow.

*Progressive changes in the human electroencephalogram under low oxygen tension.*[1] P. A. DAVIS (by invitation), H. DAVIS and J. W. THOMPSON (by invitation). Department of Physiology and Fatigue Laboratory, Harvard University, Boston, and Metropolitan State Hospital, Waltham, Mass.

Eleven normal men breathed gas mixtures containing 7.8 to 11.4 per cent oxygen, while simultaneous electroencephalograms (EEG) were recorded from occiput and from vertex. The men recorded on a writing-pad their subjective changes.

Three types of reaction were observed: (1) Increase in respiratory activity (2) Increase in pulse-rate. (3) Little or no change, either respiratory or cardiovascular. The first is the most and the third the least effective method of combatting the anoxemia. The typical sequence of changes in the EEG was: (1) Average voltage increased slightly and alpha (10/sec.) waves appeared in those records which originally showed none. (2) Alpha voltage decreased, trains of alpha waves became shorter, and

[1] Aided by a grant from the Josiah Macy, Jr. Foundation.

intervals between trains lengthened. (3) Groups of waves at 8 and 7/sec. appeared at the vertex while 10/sec. waves continued at the occiput. (4) Irregular "delta" waves (0.25 sec. or longer) appeared at the vertex and almost immediately thereafter at the occiput alternating with the 10/sec. waves. At this stage slight cyanosis was observed and subjective changes were first reported. Alpha and delta waves both ceased when the subject opened his eyes. When subjects were writing (with eyes closed) the delta waves were much reduced, but alpha waves remained. Misspellings and omissions appeared in the writing. (5) After 10 or 15 minutes of 8 per cent oxygen, when cyanosis was very marked and delta waves dominated the record, the subjects wrote slowly and illegibly. Three subjects lost consciousness. Two of the three showed large deltas, one a rather flat record, when unconscious. (6) The delta waves disappeared immediately with the first breath of room air. The alpha waves soon reappeared at their original frequency but reduced voltage. The original pattern was not fully restored for at least 2 minutes.

The sequence of changes in the EEG differed significantly from that of sleep in that no slowing of the alpha rhythm was seen.

*Oestrous behavior in guinea pigs without neocortex and other portions of forebrain.* EDWARD W. DEMPSEY[1] (by invitation) and D. MCK. RIOCH. Department of Anatomy, Harvard Medical School, Boston.

Lesions were made involving the cortex and other parts of the forebrain in a series of ovariectomized guinea pigs. Later, the animals were injected with suitable quantities of oestrin and progesterone, a procedure which invariably is followed by mating behavior in spayed animals.

Typical oestrous behavior has been observed in animals with unilateral destruction of occipital cortex and hippocampus, and unilateral destruction of neocortex, hippocampus and caudate-putamen. Likewise, sexual behavior has been observed after bilateral destruction of occipital cortex and hippocampus, and bilateral destruction of neocortex, hippocampus and caudate-putamen. Mating behavior has also been observed following destruction of the septal nuclei and anterior commissure and varying degrees of damage to fornix, tuberculum olfactorium and preoptic area.

*Dialysis of pituitary extracts.* O. F. DENSTEDT, D. K. O'DONOVAN and A. H. NEUFELD (introduced by J. B. Collip). Department of Biochemistry, McGill University, Montreal.

A preliminary study on the diffusibility of the active principles of anterior lobe extracts has shown that the thermostable metabolic stimulant passes readily through cellophane. The dialysate has been shown to possess at least three physiological properties, namely (1) it raises the oxygen consumption and depresses the R. Q. of the rabbit; (2) produces ketosis in the rat; (3) produces expansion of the melanophores in the frog.

*Studies on the vomiting reflex in cats and dogs.* A. J. DERBYSHIRE (by invitation) and J. K. W. FERGUSON. Department of Anatomy, Wayne University Medical School, and The Department of Physiology, The Ohio State University.

[1] National Research Fellow in the Natural Sciences.

Quantitative studies of vomiting in response to stimulation of the ventral vagus trunk have been made on the decerebrate cat and dog. The characteristics of the reflex can be kept at a constant level for several hours if deterioration of the peripheral nerve and the medullary centers is prevented, the former by the use of special electrodes which can be applied without dissecting the nerve or injuring its blood supply, and the latter by minimizing vascular damage during decerebration.

The pattern of the response corresponded exactly with the classical descriptions of vomiting. In our preparations it was easy to observe that the cardia consistently opened several seconds before retching began.

Vomiting was elicited with stimuli developed by an inductorium driven by two dry cells. A frequency of 10–20 break shocks per second was obtained by weighting the vibrator. In dogs a coil distance of 5.5 cm. and a period of summation of about 12 seconds produced vomiting with great regularity. In cats a coil distance of 6 cm. and a 15 to 40 second period of summation was usually adequate. In dogs recovery of excitability after vomiting was apparently complete within one minute, as evidenced by a normal period of summation when a stimulus well above threshold was used. With stimuli near the threshold a prolongation of the period of summation was observed 2 to 12 minutes after a previous vomit. This depression of excitability most marked some minutes after a previous response was observed even more strikingly in cats.

Other influences affecting the excitability of the reflex which have been investigated to date include effects of ether, dial, $O_2$ lack (9 per cent $O_2$) and $CO_2$ excess (6–8 per cent). All these have a depressing effect. No stage of augmentation under any of these influences has been observed, as yet.

Whenever apneustic breathing appeared as it usually did at a certain stage in the deterioration of the preparation, vomiting could no longer be elicited.

*The effect of deuterium oxide on choline-esterase activity.* VIRGINIA C. DICKERSON (introduced by H. G. Barbour). Laboratory of Pharmacology and Toxicology, Yale University School of Medicine, New Haven.

An electrometric titration method employing quinhydrone electrodes was used for the study of choline-esterase activity. The index of activity was the time required for the hydrolysis of 180 gamma of acetylcholine, as indicated by the neutralization of 0.1 cc. of 0.01 N sodium hydroxide, beginning and ending at pH 7.5. Auto-hydrolysis was similarly determined.

The presence of choline-esterase was found to speed the hydrolysis of acetylcholine nine and one-half times. Deuterium oxide inhibited both choline-esterase activity and auto-hydrolysis to an extent dependent upon the concentration of deuterium oxide present. The average inhibition in both cases was as follows: 20 per cent deuterium oxide, 10.6 per cent inhibition; 50 per cent deuterium oxide, 23.0 per cent inhibition; 75 per cent deuterium oxide, 33.3 per cent inhibition. These results may be explained in terms of the lower energy content of deuterium oxide.

*Rate of absorption of the basic amino acids as a function of the amount fed.* J. R. DOTY (by invitation) and A. G. EATON. Department of Physiology, Louisiana State University Medical Center, New Orleans.

In our earlier work with lysine and histidine and also with arginine, we

found individual variations in absorption rates which appeared to be definitely correlated with the amount of the compound fed. As a consequence we have extended our observations over a wider range in order to test this possibility. Our results substantiate this idea.

If we plot the rate of absorption of any of the basic amino acids against the quantity of the compound fed per unit of body weight, we find a definite tendency for the points to fall along a straight line. Of the 3 curves thus obtained, the greatest slope is displayed in the case of histidine and the least in the case of lysine. We must conclude, therefore, that in the case of each of these 3 acids the absorption rate is a direct function of the amount fed, and that the rate increment per unit increase in dosage is greatest with histidine and least with lysine. Arginine occupies an intermediate position.

Since there is no obvious reason for the existence of such a marked difference in the behavior of the basic amino acids on the one hand and glycine on the other, we felt that it was highly desirable to extend the earlier observations on the latter compound. Our experiments corroborate those of Wilson, and Wilson and Lewis in demonstrating that the quantity of glycine fed has no appreciable effect upon the rate at which it is absorbed from the gastrointestinal tract of the rat.

Histidine, arginine and lysine are absorbed from the gastrointestinal tract of the rat at rates which become greater as the amounts administered are increased. This phenomenon is not observed when glycine is fed at different levels.

*Further studies on the pulse wave in the aorta and its branches.* PHILIP DOW (by invitation) and W. F. HAMILTON. Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

Previously reported studies on the arterial pulse have been continued. Most of the experimental work has been done on dogs, and this has been supplemented by the study of records taken from humans. Optical recording from fast membrane manometers has been used throughout. The cannulae have been hypodermic needles in some cases; in others, long sections of needle tubing, entering carotid, femoral, and subclavian arteries. Simultaneous records from different portions of the arterial tree permit an extension of exact knowledge of the transformations undergone by the pulse wave and of the factors contributing thereto.

Previously reported results on the augmentation of the pulse pressure between aortic root and femoral artery have been confirmed using a cannula with side openings, demonstrating that end pressure is a negligible factor.

Analysis of transmission times shows an increase in the velocity of the pulse wave from the aortic arch to the femoral artery which is a linear function of the distance from the heart. Measurements of velocity and of the standing waves are compared with determinations of volume elasticity and of arterial dimensions; and their significance in connection with the concept of the elastic reservoir of the central arteries is discussed.

*Studies on the physiology of lipocaic.* LESTER R. DRAGSTEDT, PAUL B. DONOVAN (by invitation), W. CARTER GOODPASTURE (by invitation) and WILLIAM A. GEER (by invitation). Department of Surgery of the University of Chicago.

In March, 1936, L. R. Dragstedt, Van Prohaska and Harms reported before this society that the beneficial effect of the oral administration of raw pancreas, in preventing and relieving the fatty changes that develop in the livers of depancreatized dogs treated with insulin and in making possible the survival of these animals, was not due to its content of pancreatic enzymes or of choline but was due to the presence in pancreas of a specific substance, believed to be a new hormone The name, lipocaic, was suggested for this substance. A fat-free fraction has been obtained from beef pancreas by alcoholic extraction, which prevents and relieves the fatty changes in the livers of depancreatized dogs in a dose of 1 gram per day per os and which contains less than 5 mgs. of free choline per gram of dry substance. The effect of diets containing varying concentrations of fat, carbohydrate, and casein on the incidence of fatty changes in the livers of depancreatized dogs was studied. Diets rich in casein did not prevent the development of fatty livers but delayed their appearance in a few cases. The beneficial effect of lipocaic could not be accounted for on a dietary basis. The effect of the oral administration of lipocaic on the blood lipids was found to be inconstant in the individual case. In general, the lipids rose to about twice the normal concentration during the first week or two after pancreatectomy and then gradually decreased, reaching a level usually below the normal in 4 to 6 weeks. At this time a more or less fatty liver was commonly present. The administration of lipocaic at this time caused a rise in the blood lipids to about twice the normal level for a varying period of a week or two and then a gradual return to the normal concentration.

*Electrocardiograms of the cat during the systemic action of strophanthidin.*[1]

M. DRESBACH. Department of Physiology, Medical School, University of Pennsylvania, Philadelphia.

A search through an extensive literature failed to reveal any electrocardiograms taken during the systemic action of glucoside genins. In the work here reported, such records, with controls, were taken by one (usually L II) or all of the three customary leads on 14 normal, intact cats, 20 under moderate amytal anaesthesia and on 6 about two hours after double cervical vagotomy and bilateral excision of the stellate ganglia, under ether (acute experiments). In each instance the cats received 0.20 to 0.30 mgm. per kgm. of strophanthidin intrahepatically or by some other route. The dose was occasionally repeated.

Practically all of the changes in electrocardiograms (taken by the leads mentioned), including the so-called characteristic ones in the RS-T segment and T-wave, known to result from action of the digitalis bodies were obtained. It was observed that many of these effects occurred in all of the three groups mentioned and that most of them rapidly and completely disappeared; certain of them persisted for a relatively long time in an occasional cat. In nearly all instances the experiment could be repeated ad libitum with an individual animal, without harm to it, if two or three days were allowed between injections. The alterations in the electrocardiogram apparently bore no definite relation to the onset of vomiting or other responses to strophanthidin.

[1] The above work was done in the Department of Physiology, Albany Medical College, Albany, N. Y.).

*An evaluation of the Frank method for the determination of urinary prolan in nonpregnant women.* DELLA G. DRIPS (by invitation), A. E. OSTERBERG, GERTRUDE FISHER (by invitation) and KATHLEEN LEWIS (by invitation). The Mayo Clinic and Mayo Foundation, Rochester, Minn.

An investigation has been made to evaluate the quantitative determination of the urinary prolan is nonpregnant women. The method used for the extraction has been described by Frank, Salmon and Friedman. Albino rats of the Wistar strain were used in the biological assays. The uteri and ovaries were observed grossly and weighed, some at twenty-two hours, and others at ninety-six hours, after the first injection. The ovaries were then studied microscopically. The microscopic change in the ovaries at ninety-six hours appears to be the most reliable criterion for a quantitative estimation of the urinary prolan by this method.

Two hundred and ninety-two determinations were made on the urine of 248 women. Of these 235 presented a variety of clinical conditions, which we thought might be associated with abnormal gonadotropic function of the anterior lobe of the pituitary, and 13 who appeared to be normal were chosen as controls.

It is not always possible to demonstrate prolan in the urine of normally menstruating women. We have not found it present in amounts higher than 15 rat units per liter. When it is present in amounts over 20 rat units, the women may, at any age, present menstrual irregularities and associated clinical symptoms. These include vasomotor disturbances similar to those of the true menopause period during which the values for prolan run as high as 66 rat units per liter. The degree of severity of the symptoms seems to correspond to the amount of prolan found in the urine.

*Further studies of factors influencing brain rhythms.* H. DUBNER (introduced by R. W. Gerard). Department of Physiology, University of Chicago, Chicago, Illinois.

The influence of brain polarization and altered blood ion concentration upon the optic rhythm in the cat's lateral geniculate body have been reported (Dubner and Gerard). Additional experiments dealing with the influence of blood sugar level, midbrain transection, and degree of dark adaptation upon these potentials are here presented. As before, a concentric electrode was placed with the Horsley-Clarke instrument and the amplified potentials recorded with a cathode-ray tube. Nembutal (35 mgm. per kgm.) was administered intraperitoneally.

Insulin (up to 15 units per kgm. subcutaneously) did not give gross convulsions, though potential changes were definite within an hour. The small high-frequency potentials superposed on the usual optic rhythm were accelerated and intensified; and the normal "on" response to light was replaced by a series of three to five large oscillations, mainly positive, and followed by a prolonged high-frequency after-discharge. The altered response remained constant with repeated stimuli. The normal picture was restored within five minutes after intravenous injection of glucose (2.0 cc. 40.0 per cent).

Complete midbrain transections, four experiments, increased the amplitude of the optic rhythm (3/sec.) from a previous 20 to 40 $\mu$V and made it more regular. In addition, a strikingly rapid rhythm of more than 25/sec. and 25 $\mu$V amplitude was added to the slow one. These modified rhythms

persist for hours; are almost completely inhibited during light and return following it; and are depressed to one-fourth within one minute after injecting additional Nembutal (15 mgm. per kgm.) to gradually regain amplitude as the anesthetic wears off.

In four experiments in which the optic rhythm had disappeared in continued darkness, moderate light stimulation reinitiated it. The discharge continuing after a light has been flashed can also be modified by preceding optic stimulation. Thus in two experiments this after-discharge lasted 15 sec. for a flash after a period of darkness and 35 sec. for an identical flash given after three preceding ones. Incidentally, such after-discharges may show a regular alternation, at about 1 sec. intervals of wave trains at 6 and at 80 a second.

These observations bear on the question of control of neurone rhythms by impinging impulses and by metabolic factors.

*Conditioning neurosis in the dog and cat.* SIMON DWORKIN. Department of Physiology, McGill University, Montreal, Canada.

Permanent disturbance of conditioned behavior was seen by Pavlov in dogs that had been trained to make difficult discriminations. The condition was attributed to a final breaking down of the balance between excitatory and inhibitory processes.

During several years, we have noted some interesting species differences between the dog and the cat when placed under similar conditions of nervous stress. In the cat inhibition, while not difficult to establish, was always weaker than in the dog, and more easily abolished. To extraneous stimuli the cat was curiously indifferent, as a rule. Spontaneous sleep, occasionally seen in the dog, was never seen in the cat. When the cat was trained to make difficult discriminations, it soon "escaped" by responding positively to both positive and hitherto negative stimuli.

While the cat was found to be immune to the discrimination neurosis of the dog, a marked, though short-lasting disturbance could be produced by training to sound stimuli of threshold value. As the loudness of the tones was reduced the animals, instead of waiting for the signals, showed bursts of spontaneous activity (interval responses) which made further testing impossible. Sometimes normal behavior could be re-established by presentation of several successive loud tone stimuli; at other times some days' rest was needed. In three cats the abnormal behavior lasted for three weeks.

*Utilization of sodium acetoacetate by dogs and cats under various experimental conditions.* J. A. DYE and JANE L. CHIDSEY (by invitation). Department of Physiology, Cornell University, Ithaca, N. Y. (Read by title.)

Sodium acetoacetate was injected intravenously into amytalized nephrectomized dogs and cats. Curves of total blood acetone and blood glucose utilization were plotted. Injections of glucose during the experiments, although producing sharp rises in blood sugar, did not alter the blood acetone curve nor in any way increase the total acetoacetate utilized. Similarly, the injection of large doses of acetoacetate into eviscerated animals did not increase the rate of glucose utilization. Removal of the liver and abdominal viscera does not decrease, but may increase the tol-

erance of dogs for the acetoacetate. These results confirm those previously reported by others. The tolerance of hypophysectomized dogs and cats for acetoacetate is within the normal range.

*Utilization of sodium acetoacetate*

N = normal; HE = hypophysectomized animals. Average figures

| ANIMALS | NUMBER USED | EXPERIMENTAL CONDITIONS | BLOOD GLUCOSE | TOTAL BLOOD ACETONE | ACETOACETATE UTILIZATION, FIRST HOUR |
|---|---|---|---|---|---|
| | | | *mgm. per cent* | *mgm. per cent* | *per cent* |
| N dogs | 2 | Eviscerated | 36.4 | 165.5 | 61.4 |
| N dogs | 5 | Same, with glucose injections | 131.8 | 146.8 | 61.7 |
| N dogs | 3 | Nephrectomized only, no glucose | 80.0 | 116.0 | 46.6 |
| HE dogs | 1 | Nephrectomized only, no glucose | 41.0 | 175.0 | 41.0 |
| HE dogs | 2 | Same, with glucose injections | 108.0 | 165.0 | 42.6 |
| HE cats | 5 | Same, with glucose injections | 199.0 | 199.0 | 40.5 |
| N cats | 4 | Same, without glucose injections | 181.0 | 140.0 | 39.6 |

*Studies on the heat production and blood and urine constituents after administration of d-arginine monohydrochloride to the dog.* A. G. EATON and J. R. DOTY (by invitation). Department of Physiology, Louisiana State University Medical Center, New Orleans.

The dogs used in this work were fasted at least 16 hours before the experiment. Several determinations of basal metabolic rate and blood amino and urea nitrogen values were made in a control period preceding the administration of the arginine. Blood urea and amino nitrogen levels as well as heat production were determined hourly in a 6 hour experimental period. Heat production was measured by the Tissot Haldane method, urea by the manometric urease method of Van Slyke, and amino nitrogen by the manometric method of Van Slyke.

In some experiments, 10 grams of arginine were administered intravenously and in others by the oral route. There is a prompt and rapid rise in the blood of both amino and urea nitrogen. Following intravenous administration the amino nitrogen returns to the control level in about 3 hours, while after oral administration the value at the end of the 6 hour period is somewhat elevated. Blood urea nitrogen levels are increased in both cases and remain high throughout the period, although the rise is slower after oral administration.

Following intravenous injection, 3–4 grams of the arginine monohydrochloride appears in the urine but much less is excreted after ingestion. There is a considerable increase in urine urea nitrogen although less than with glycine.

Arginine exhibits a specific dynamic action which is less than that of glycine. It is metabolized somewhat more slowly than glycine but much more rapidly than lysine.

*Further observations on the calcium appetite of parathyroidectomized rats.* JOHN F. ECKERT (introduced by Curt P. Richter). Psychobiological Laboratory, Phipps Clinic, Johns Hopkins Hospital, Baltimore, Md.

Previous experiments have shown that when fed calcium-free McCollum food and given free access to a calcium lactate solution and water, parathyroidectomized rats show a greatly increased appetite for calcium. In the present experiments the appetite for other calcium salts and for salts of sodium, potassium, and ammonium was studied. When given a choice of water and calcium gluconate (3.3 per cent), parathyroidectomized rats showed a greatly increased appetite for the calcium solution. The same was found for calcium acetate (1 per cent). Nineteen parathyroidectomized rats, each given a choice of sodium chloride (3 per cent), sodium phosphate (8 per cent), sodium iodide (1 per cent), calcium lactate (2.4 per cent), potassium chloride (1 per cent), and ammonium sulphate (1 per cent), all showed a greatly increased calcium intake. In some of the animals the intake of sodium chloride and of potassium chloride was also increased, but to a less extent than for the calcium lactate. The intake of sodium phosphate either remained unchanged or decreased after operation. The intake of sodium iodide and ammonium sulphate remained unchanged. Further evidence is thus presented for the selective appetite of parathyroidectomized rats for calcium.

*The excretion of sodium ferrocyanide and phenol red by the normal and mercurialized frog and rat.* J. GRAHAM EDWARDS. School of Medicine, University of Buffalo, Buffalo, N. Y.

The evidence is substantial that mercuric chloride injures the renal corpuscle considerably less than parts of the proximal convolution. On the basis of this evidence, excretion via the corpuscle should be less affected by the action of mercury than via the proximal convolution. In order to test this possibility, the excretion of two substances was quantitatively determined. One of these substances, Na-ferrocyanide, is believed to be excreted chiefly by the corpuscle; the other, phenol red, by the proximal convolution. The results indicate in the frog that Na-ferrocyanide may be excreted largely by the proximal convolution and phenol red by the corpuscle. In the rat, the excretion of Na-ferrocyanide seems to be chiefly via the corpuscle and phenol red via the proximal convolution.

*The relation between configuration of the heart and certain electrocardiographic findings.* I. L. ERSHLER and L. M. HICKERNELL (introduced by J. S. Robb). Department of Pharmacology and the Student Health Service, Syracuse University.

The Student Health Service at Syracuse University has initiated a long time program having to do with the study of certain abnormal phenomena in individuals of college age presenting no altered physical signs and no symptoms. It is hoped in the course of this work to discover earlier manifestations, possibly the beginnings of certain types of degenerative disease. In the course of such routine study it was noted that certain individuals, usually of asthenic structure, had electrocardiograms with low voltage in Lead 1 only, while those of Pyknic structure exhibited low voltage in Lead 3 only. Examination of roentgenograms of these two types of individuals reveals that the configuration of the hearts offers a

physical explanation for the findings. This is reasonable in view of the fact that the cardiac action current in a ptotic heart becomes more nearly perpendicular to the direction of lead 1 and more nearly parallel to the direction of lead 3. The reverse holds true for the transverse type of heart. We are grateful to Dr. J. S. Robb for directing and providing technical assistance for this study. (Lantern slides.)

*Blood pressure and coronary blood flow during muscular exercise in the dog.* HIRAM E. ESSEX, JULIA F. HERRICK, EDWARD J. BALDES and FRANK C. MANN. Mayo Clinic and Mayo Foundation, Rochester, Minn.

Previously we have reported the changes in blood flow in one of the coronary arteries accompanying exercise on a treadmill. As a rule the coronary blood flow increased rapidly with the beginning of exercise and subsequently each time the exercise was augmented. In each case the blood flow soon decreased after the initial rise but remained greater than the flow observed before the additional exercise was begun. The flow became relatively constant at the new level until the rate of exercise was altered. It was thought probable that an explanation of these observations would be found in a study of the blood pressure during exercise. Therefore the effect of exercise on the blood pressure in the femoral artery of a series of animals has been recorded by the standard method (cannulation, mercury manometer and kymograph). In addition simultaneous records of femoral blood pressure and coronary blood flow have been made on a small series of dogs. The alterations in blood pressure during exercise have not been of a nature to explain adequately the observed changes in coronary blood flow resulting from exercise. There was considerable variation in the response of the blood pressure of different animals to exercise but in general there was a definite rise in blood pressure when exercise was begun or when the degree of exertion was increased but the increased pressure was usually maintained and in some experiments the blood pressure continued to increase throughout the period of exercise.

*The preparation and metabolism of hydroxypyruvic aldehyde.* WILLIAM E. EVANS, JR., C. JELLEFF CARR and JOHN C. KRANTZ, JR. (introduced by Wm. Amberson). Department of Pharmacology, School of Medicine, University of Maryland, Baltimore.

Hydroxypyruvic aldehyde was prepared by a modification of the Evans and Waring process in which the excess of copper was removed by means of oxalic acid. The product was free from copper and oxalate. The fate of this compound in the animal body was studied. It tends to decrease stored liver glycogen in the white rat. Although it produces no significant hyperglycemia within an hour in the rabbit when administered by vein, it causes a gradual rise in blood-sugar level when given perorally. Insulin shock produced in the mouse was relieved temporarily. The respiratory quotient of the male white rat was raised, apparently by direct catabolism. Normal and neoplastic tissue response to its presence was also determined from the standpoint of pH and lactic acid formation.

*Spread of impulse over the ventricles in the mammalian heart.* J. A. E. EYSTER, WALTER J. MEEK and WARREN E. GILSON (by invitation). University of Wisconsin, Madison.

With two Clement differential electrodes connected through amplifiers to two cathode ray oscillographs, the time of onset of activity of various points on the anterior and posterior surface of the dog's heart has been determined by simultaneous recording of the action potentials. One electrode was kept fixed, the other moved to various points on the surface. The potential time curves recorded by the differential electrode under the conditions of our experiments show a constant pattern and allow accurate measurement of time intervals. They are at least relatively free from effects due to distant potentials ("extrinsic effects") as shown by the fact that when recorded from the auricle they show no evidence of ventricular potentials, and when recorded from the ventricles, no evidence of auricular potentials. The curves were measured in a comparator under 40 times magnification. With the exception of points along the interventricular septum, which are involved early, and points near or on the conus of the pulmonary artery which are involved late, the surface points on both ventricles are involved within less than a hundredth of a second of one another. All surface points are generally involved within 0.02 sec.

*The elimination of sulfanilamide by the prostate gland of the dog.* JAMES I. FARRELL, YALE LYMAN and G. P. YOUMAN (introduced by F. T. Jung). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

Dogs were selected as an experimental animal because they possess a relatively large prostate gland. One of us (Am. J. Physiol. **118:** 64, 1937) had previously devised an operation which makes collection of pure prostate secretion a simple procedure. Briefly, the internal urethral orifice is closed with a silk ligature. The urethra distal to the prostate is divided between clamps, and a glass cannula inserted into the prostatic urethra. Each vas deferens is ligated. By injecting 1 to 2 cc. of one per cent pilocarpine intravenously, a prompt and copious secretion of the prostate occurs; as much as 150 cc. of secretion may be collected in 3 hours.

To animals prepared by the above method, we gave sulfanilamide by mouth or intravenously in various dosages, the average daily dose being 0.18 gram per kilo of body weight. The concentration of sulfanilamide in blood, prostatic secretion, and urine was determined by a colorimetric method. When large doses were given at four hour intervals, the concentration of sulfanilamide was 15 mgm. per 100 cc. of prostatic secretion.

The secretion containing sulfanilamide was collected under sterile precautions and placed in tubes which were inoculated with a 4 mm. loopful of broth culture of either Staphylococcus aureus or B. coli. Because of the delicate nature of gonococci and the ease with which they are killed in cultures, the more resistant bacteria were used.

The sulfanilamide excreted in the prostatic secretion was sufficient to kill B. coli in 24 hours in all cultures. The numbers of organisms were reduced in two and four hour plates. The drug was not as effective against Staphylococcus aureus, unless given intravenously.

These experiments demonstrate that the drug is excreted from the prostate gland into the posterior urethra in concentrations sufficient to inhibit growth of bacteria. Thus, the secretion of sulfanilamide by the human prostate would tend to prevent the spread of anterior urethral infections, notably gonococcal infections, into the posterior urethra.

*The oxidation of various substrates by the diabetic kidney.* J. F. FAZEKAS (by invitation), Z. BAKER (by invitation) and H. E. HIMWICH. Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y.

The effects of various substrates on the respiratory quotient were studied on kidney excised from depancreatized cats. Slices of renal cortex obtained from animals three or more days depancreatized were studied in a Warbur respirometer, saline buffered with phosphate being the fluid medium. Normal renal cortex is capable of oxidizing glucose. However, this is not true of depancreatized kidney, which yields neither an increase of oxygen consumption nor a rise in respiratory quotient in the presence of glucose. Thirteen observations, ranging from 0.62 to 0.74, yielded an average respiratory quotient of 0.67. With succinate the oxygen consumption rose markedly, while the respiratory quotient fell. These results in large part may be attributed to the oxidation of the succinate to fumarate. In 9 observations, using succinate as substrate, the respiratory quotients varied between 0.26 and 0.53 with an average of 0.42. In 3 observations, glucose and succinate together exhibited respiratory quotients of 0.45, 0.48, and 0.49. Fumarate, on the other hand, gave respiratory quotients which varied from 0.76 to 0.95, and averaged 0.86. Pyruvate (9 observations) similarly exhibited an increase in the respiratory quotient, varying from 0.81 to 0.94, and averaging 0.88. These data indicate that the diabetic kidney is capable of oxidizing to some extent various intermediates in the metabolism of glucose.

*A quantitative study of calcium and cephalin in relation to thrombin formation.* JOHN H. FERGUSON. Department of Pharmacology, University of Michigan, Ann Arbor.

A standardized technique has been developed for the quantitative study of the factors involved in thrombin formation (in vitro). The reagents are controlled for "purity" and stability. They include (1) calcium salts, (2) cephalin, (3) prothrombin (poor in cephalin), and (4) fibrinogen (prothrombin-free). Amongst controlled physical variables may be listed (a) dilution (total), (b) concentration (individual factors), (c) pH, and (d) temperature.

The experimental unit is the prothrombin activation (thrombin formation) curve obtained by plotting the clotting-time of test samples against the age of the thrombin mixture. Incubation at 38°C. speeds up the activation at the expense of some loss in activity (especially in the post-optimal period). This is due to metathrombin formation. At 15°–20°C. the thrombin is stable.

Keeping the prothrombin and calcium concentrations fixed, the cephalin factor is varied. Lessening the cephalin quantitatively decreases the rate of thrombin formation. The test system is sufficiently sensitive to detect variations of 0.0005 mgm. of cephalin. Increasing the cephalin to "excess" may expose an inhibitory phenomenon attributable to "free acidity" (cf. pH factor).

Keeping the prothrombin and cephalin constant, the calcium content is modified. Marked diminution of activity results from a calcium-ion decrease. Excess of calcium inhibits.

Tests at 20°C. suggest that there is a true difference in the optima at

low calcium and cephalin concentrations, indicating a quantitative influence not only upon rate of activation, but also upon the amount of thrombin produced.

Varying both the calcium and cephalin demonstrates the absence of any controlling influence directly attributable to (a) the Ca:Cephalin ratio, or (b) the amount of calcium-cephalin compound calculated to be present.

It is concluded that thrombin formation depends primarily upon the quantity of calcium in relation to the prothrombin factor (this important variable being fixed). The cephalin is necessary, however, for the union of calcium with the prothrombin. The "intermediary substance" in thrombin formation (Ferguson: Am. J. Physiol., 1937, **119:** 755) is, therefore, a colloidal complex of prothrombin *plus* cephalin *plus* calcium.

*The distribution of $CO_2$ between erythrocytes and plasma in dogfish blood.* J. K. W. Ferguson, S. M. Horvath (by invitation) and J. R. Pappenheimer (by invitation). The Marine Biological Laboratory, Woods Hole, and The Department of Physiology, The Ohio State University, Columbus.

The distribution of $CO_2$ and of chloride between erythrocytes and plasma has been determined in the blood of some twenty-five smooth dogfish (Mustelus canis) and two spiny dogfish (Squalus acanthius). The distribution ratios, $\gamma CO_2$ and $\gamma Cl$ represent the ratios of intracellular to extracellular $CO_2$ and Cl respectively, the concentrations being expressed per unit $H_2O$ in each phase. Values of $\gamma CO_2$ were usually calculated from determinations of $CO_2$ in whole blood and its true plasma. Since under physiological conditions the total $CO_2$ is low in these bloods, rather large errors are to be expected in individual values of $\gamma CO_2$. For this reason only mean values of $\gamma CO_2$ for comparable groups of determinations are reported. The means for six such groups varied from 1.0 to 1.9. Thus it appears that in these bloods the concentration of $CO_2$ in the erythrocytes is usually much higher than in the plasma, a situation very different from that found in mammalian blood and not easily reconciled with view that the bulk of the combined $CO_2$ in the cell is in the form of active bicarbonate ions. Values of $\gamma Cl$ on the other hand varied from 0.45 to 0.9, values which are as low or lower than those for mammalian blood.

The contrast with mammalian blood is even more striking when the ratios of $\gamma CO_2$ to $\gamma Cl$ are considered. In mammalian blood this ratio has a value of about 1.25. In these dogfish bloods the ratios varied from 1.8 to 2.5. A substantial portion of the discrepancy between $\gamma CO_2$ and $\gamma Cl$ in mammalian blood can be attributed to the presence of carbamino compounds of $CO_2$ in the red cell. Such compounds may to some extent explain the excess $CO_2$ in dogfish erythrocytes. As yet however, no estimate can be made of the probable amount of carbamino $CO_2$ in dogfish blood. Attempts to measure the carbamino compounds of $CO_2$ with fish hemoglobin have been frustrated by a number of peculiar difficulties.

*A shaking machine for blood pipettes.* R. L. Ferguson (by invitation) and R. D. Templeton. The Departments of Pathology-Bacteriology and Physiology of Loyola University, School of Medicine. (Demonstration.)

The apparatus consists of a suction driven motor enclosed in a metal casting, and a carriage for holding 6 hemocytometer pipettes.

The motor is operated by a suction pump fastened to a water faucet which delivers a minimum of 25 pounds of pressure. The speed of the motor, which can be varied within limits, has a maximum of about 80 oscillations per minute.

The rubber covered carriage which measures 9 by 7 cm. is attached by a metal beam to a rocker arm. This attachment (held by a set-screw) is adjustable so that the pipettes may be carried through an arc either at right angles to or in the direction of their long axes. The angle of swing is approximately 80°. This carriage is interchangeable with other carriages that may be built for holding small vessels of various designs.

*Relation between basal metabolism and summation of excised tissue metabolism in young albino rats.* J. FIELD, 2nd, H. S. BELDING (by invitation) and A. W. MARTIN (by invitation). Department of Physiology, Stanford University, Calif.

Whether the basal metabolic rate of mammals (in terms of oxygen consumption) is merely the sum of the rates of oxygen consumption of the several component tissues, or whether some controlling influence is exerted by the organism as a whole, is still a moot point. The experiments here reported were designed to throw some light on this question in the case of the young albino rat. Thirty-three animals (15 males, 18 females), of the Slonaker-Wistar strain, ranging in age from 100 to 120 days, were used. The animals were maintained on the Sherman "optimal" diet.

Basal metabolic rates were determined on each animal in the post-absorptive (18 hour) state by a method allowing correction for activity. The animal was then sacrificed and the rate of oxygen consumption of the following tissues determined by the Warburg manometric method at 37 $\pm$0.01°C. The figures in parentheses give the mean value of the oxygen consumption of the preceding tissue, in cc. N.P.T. per gram wet weight per hour, extrapolated to the time of excision (For each individual, four or five tissues were studied). Skeletal muscle (diaphragm 1.75, semimembranosus anticus 0.97, external oblique 0.70, trapezius 0.96); smooth muscle (small intestine 1.05); cardiac muscle (ventricular wall 1.41); brain (cortex 1.95, medulla 1.63); kidney (4.12); liver (1.97); spleen (1.47); lungs (1.35); skin (0.31); bone (0.23); testis (1.01). These values were summated using Donaldson's factors for organ weights (Wistar tables). The oxygen consumption so obtained for the average animal totalled about 66 per cent of the average (intact animal) B.M.R. (0.728 cc. N.P.T. per gram per hour and 1.098 cc. N.P.T. per gram per hour respectively). This finding suggests that the B.M.R. of the young albino rat consists simply of the sum of the rates of oxygen consumption of the several tissues plus the minimal functional activity of the basal state.

*The excretion of endogenous "creatinine" by the human kidney.* THOMAS FINDLEY, JR. (introduced by H. L. White). Washington University School of Medicine, St. Louis, Mo.

Plasma clearances of total chromogenic material in blood and urine were determined before and after ingestion of creatinine in four normal

subjects and two nephritics. The points obtained by plotting plasma concentration against urinary excretion rate fell in every case in such a manner as to indicate that when urinary excretion reached its hypothetical zero there was still about 0.5 mgm. per cent of Jaffé-reactive substance in plasma. When the clearances of total chromogenic material were arbitrarily corrected by this amount they became identical with those calculated on exogenous creatinine alone. Whereas the clearance of total chromogenic substance diminishes as normal plasma concentrations are approached, the exogenous and corrected clearances are quite independent of blood level between 1.6 and 14 mgm. per cent. This constancy of behavior is used as an argument that most of the chromogenic substance in human plasma is actual creatinine and that exogenous creatinine is not eliminated by tubular secretion.

*The birefringence of smooth muscle (Phascolosoma and Thyone) as related to muscle length, tension and tone.* ERNST FISCHER. Marine Biological Laboratory, Woods Hole, Mass. and Department of Physiology, Medical College of Virginia, Richmond, Va.

Phascolosoma and Thyone retractor muscles show marked "viscoidal tone." A muscle stretched by a load is constantly lengthening, and when the muscle is released later on, it shortens only to a small extent. By alternatingly loading and releasing a living muscle with increasing weight, the "total birefringence" of the muscle can be measured for the same muscle length under a well determined tension and under no tension. In the absence of tension the birefringence is about proportional to the square root of the length as found by Botzler for a smooth snail muscle under comparable conditions. For the same muscle length under tension the birefringence is higher, the increase being about proportional to the tension present—a true "photoelastic effect." When a muscle is stretched quickly, thus producing marked tension, the birefringence increases at a steadily growing rate until suddenly the double refraction diminishes and the muscle tears through.

Analysis of muscles fixed at various lengths with formaldehyde shows an increase of "form birefringence" about proportional to the square root of the muscle length, while the "micellar anisotropy" is more or less constant, with the exception of a marked diminution for strongly shortened muscles.

For both muscles investigated the same relations between length, tone, and birefringence has been found. But compared with Phascolosoma muscle the "total birefringence" of the Thyone retractor is only about 60 to 70 per cent and the "miscellar anisotropy" is a relatively smaller fraction of "total birefringence." These differences in submicroscopical structure may be correlated to the difference in contraction velocity and forces developed by those muscles.

*The steady state frequency of the embryo fish heart at different cyanide concentrations.* KENNETH C. FISHER and RICHARD OHNELL (introduced by Laurence Irving). Marine Biological Laboratory, Woods Hole and Department of Biology, University of Toronto, Canada.

When an embryo fish is exposed to neutral solutions of cyanide the heart beat frequency falls, the time course of the fall being that of a first order

process, to a level from which it subsequently falls only much more slowly. We have investigated the relation between this inhibition level and the cyanide concentration.

In Fundulus embryos at 23° $\frac{N}{1000}$ cyanide produces a maximum effect which amounts to a lowering of the frequency on the average to 27 per cent of the normal. Of the 73 per cent of the frequency which is cyanide-sensitive, the fraction which is affected at any particular cyanide concentration is related to the cyanide concentration by an expression of the mass action law. This is the result to be expected if the cyanide-sensitive portion of the frequency is directly proportional to the oxygen consumption by the chemical mechanism involved, and if, in that mechanism, cyanide combines with an essential catalyst in stoichiometric proportions. A preliminary set of observations on Fundulus embryos at 15°, and on trout embryos at 5° and 15° suggest that the maximum inhibition produced by cyanide increases at higher temperatures.

Currently the lack of complete inhibition by cyanide, as well as any change in the cyanide-sensitivity of a system, is considered to be due either (1) to the possibility that respiration does not proceed entirely through Warburg's *atmungsferment* or (2) to a change in the "affinity" of the respiratory catalyst for cyanide. The experiments described do not indicate which of these possibilities is the case though provisionally we regard the former as the more likely interpretation. If this view be correct then the data show that the cyanide-sensitive system has a higher temperature coefficient than that portion of the system which is insensitive to that inhibitor.

*The tubular reabsorption of glucose in the dog.* SAUL FISHER (by invitation) and JAMES A. SHANNON. Department of Physiology, New York University College of Medicine, New York City.

The tubular reabsorption of glucose has been examined in the unanesthetized dog at arterial plasma concentrations varying from the normal to 1800 mgm. per cent. As the plasma concentration of glucose is raised the tubular reabsorption increases proportionally and is essentially complete until a maximal rate is reached, after which any excess glucose in the glomerular filtrate is excreted. It is thus a critical rate of filtration of glucose in conjunction with its maximal rate of reabsorption, rather than a critical plasma concentration, that constitutes the essential feature of the threshold mechanism. In the same animal on a constant diet the maximal rate of reabsorption may be quite constant over long periods of time (6 months). This maximal rate is demonstrable in decerebrate dogs over a range of 400 to 2400 mgm. per cent. When the renal blood pressure, and hence the rate of filtration, is lowered by the application of a clamp to the abdominal aorta, there is no significant decrease in the value of the maximal rate of reabsorption; this excluded the possibility that the diffusion gradient of glucose established by reabsorption is a factor limiting the reabsorptive process. The above facts indicate that a limitation in the energy available for glucose transfer is not, at least in the normal animal, a primary limiting factor.

We believe the essential limitation in the reabsorptive system to be similar to the one suggested to explain a similar maximum in the rate of tubular excretion of certain substances, where it is posited that the

solute enters into reversible combination with a cellular constituent, present in limited and constant amount, and that the rate of decomposition of this complex limits the process of transfer.

*Insulin sensitivity in endocrine disorders.* GERALD FLAUM (introduced by Elaine P. Ralli). Laboratories of the Department of Medicine and the Diabetic Clinics of the Department of Medicine, New York University College of Medicine.

The differentiation of diabetes mellitus into "insulin-sensitive" and "insulin-insensitive" types was suggested by Himsworth on the basis of the comparison of two blood sugar curves; one following the oral administration of glucose; the other following the oral administration of glucose and the simultaneous intravenous injection of insulin. Those patients in whom the injection of insulin suppressed the hyperglycemia normally following the ingestion of glucose were classified as "insulin-sensitive"; those in whom the insulin did not have this effect were classified as "insulin-insensitive."

Various types of patients and normal subjects are being studied by means of these detailed curves. The evidence at present tends to show that, with the dosages of glucose and insulin used, there is complete suppression of the hyperglycemia following oral glucose in the normal group. This also occurs in the "insulin-sensitive" type of diabetes. The curves in this group are similar to those of normals, but occur at a higher blood sugar level, which suggests that these diabetics react in the normal way to insulin and that their diabetes is due, not to an inability to use insulin, but rather to a lack of it.

In the "insulin-insensitive" type of diabetes there is an inability on the part of the body to utilize insulin. The curves after simultaneous glucose and insulin differ little, if at all, from those following glucose alone. Curves of this type were obtained in the study of diabetes associated with acromegaly and suggest by indirect evidence that hyperactivity of the pituitary gland is responsible for the "insulin-insensitivity" in this instance. The implication is that the phenomenon of "insulin-insensitivity" when seen in the diabetic uncomplicated by obvious endocrine dysfunction is due possibly to hyperactivity of one or more of the endocrine glands.

*The effect of colchicine on cell division in animals treated with hormone.* W. FLEISCHMANN (introduced by R. W. Gerard). University of Vienna, Vienna, Austria, and the University of Chicago, Chicago, Ill.

The colchicine technique (Dustin; Allen, Smith and Gardner) is generally useful in the study of growth phenomena induced by hormones. It has been used to study the action of male sex hormones on the seminal vesicle of the castrated mouse, and colchicine aids their biological assay (Mitogenesetest of Loewe and Voss). In a similar manner, the morphological changes in the thyroid of the guinea-pig, produced by administration for several days of the anterior pituitary thyreotropic hormone, are emphasized by injection of colchicine; and the assay of thyreotropic hormone so aided.

Lastly, the reaction of the bitterling ovipositor to estrogenic substances (Fleischmann and Kann) was analyzed with the help of a modified colchicine technique (larger doses of colchicine are necessary in cold-blooded

animals), which made growth zones in the ovipositor apparent. In accordance with the work of Barnes, Kanter and Klawans and of Kleiner, Weisman and Mishkind, we found that extracts of adrenal cortex as well as the pure active substance, corticosterone, lately isolated from the adrenal cortex by Reichstein, have a marked effect on the growth of the bitterling ovipositor.

*Biochemical changes associated with the onset of secretion in the fetal chorioid plexus.*[1] LOUIS B. FLEXNER and ROBERT D. STIEHLER (by invitation). Departments of Anatomy and Ophthalmology, Johns Hopkins Medical School, Baltimore.

Distribution ratios between blood plasma and cerebrospinal fluid of fetal pigs indicate that the fluid is an ultrafiltrate up to the 45th day of gestation; later it is a secretion. At this transition period the following changes have been noted in the chorioid plexus: 1. Prior to secretory activity, the oxidation-reduction potentials of stroma and epithelium are identical or nearly so. With onset of secretion, the stroma potential becomes approx. 100 millivolts more negative than the epithelium potential. The concentration of indophenol-oxidase appears equal in stroma and epithelium of presecretory stage; in the secretory stage, the oxidase is limited to the epithelium and is in increased concentration. 2. Prior to secretion selected acid and basic dyes diffuse from stroma to epithelium or vice versa and are unaffected by asphyxia. In the secretory stage, the same basic dyes pass from stroma to epithelium but do not pass from epithelium to stroma readily. The selected acid dyes pass from epithelium to stroma but not readily in the reverse direction. With asphyxia, these dyes behave as in the presecretory stage. These observations are explained on the theory that with secretion an electric current develops between stroma and epithelium and that this energy is supplied by an organization of oxidative and reductive processes.

*Changes in the lymphatic organs during the alarm reaction.* V. G. FOGLIA (by invitation) and H. SELYE. Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada.

The alarm reaction (elicited by muscular exercise, subcutaneous injections of formaldehyde, surgical shock or exposure to cold) causes marked atrophy of the thymus, the spleen and the lymph glands which are accompanied by characteristic histological alterations. These changes are very similar in the rat, guinea pig, rabbit and cat, so that they may be regarded as a general response which is not species specific. Especial attention is given to the formation of "hemolymph nodes" which has frequently been observed during the alarm reaction, irrespective of the stimulus which elicited it. The physiological significance of this hemolymph node formation is discussed.

*Studies on the activity of circular and longitudinal muscle of extra-peritonealized ileum in a human subject.* ARMAND C. FORSTER (by invitation) and ALRICK B. HERTZMAN. Departments of Surgery and Physiology, St. Louis University School of Medicine, St. Louis, Mo.

Circular and longitudinal muscle activity of an externalized loop of

[1] This investigation was supported in part by a grant from the John and Mary Markle Foundation.

lower ileum was recorded in an otherwise "normal" human subject with myographic technique. The time relations of this activity to expulsion of normal intestinal contents from the loop of externalized bowel through its visible anus were observed.

Normal activity of the lower ileum is both propulsive and non-propulsive in character.

For propulsive action (either by peristaltic wave or peristaltic rush) both muscle coats work in a series of single simultaneous contractions, with the onset of longitudinal muscle contractions preceding the onset of circular muscle contractions by ½ to 1½ minutes, and continuing after activity in the circular muscle has ceased. Efficiency of propulsion was increased when both longitudinal and circular coats contracted synchronously. These synchronous contractions, classified as peristaltic waves, occurred from 1 to 6 times during a 30 minute period with an average duration of 1 to 6 minutes.

Non-propulsive activity consists of two wave types: (1) single, or (2) short series of single contractions having an equal phase of contraction and relaxation, and predominating in the longitudinal muscle. A third wave type, probably the wave resulting in pendulum movements, exhibits a series of contractions lasting for 45 seconds or more, and producing an even gentle flow of feces from the exposed bowel. These three wave types, classified as single mixing waves, appear 9 to 150 times during a 30 minute period; each wave having an average duration of 6 to 12 seconds.

A loss in tone of both muscle coats with slow recovery to normal follows the peristaltic wave. The loss in tone and the time required for tonus recovery seemingly depend on the amplitude of the previous contraction. As the patient improved clinically, the time required for tonus recovery became less. At no time did a second peristaltic wave follow until this tonus recovery had been complete. We were unable to demonstrate a loss in tone of either muscle coat prior to the peristaltic wave.

Pharmacological observations on the preparation are being reported elsewhere.

*Respiratory failure in acute nicotin poisoning.* F. E. FRANKE. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

By means of a modified cross circulation experiment it was possible to allow nicotin to gain free access to the head only or in other experiments to the trunk only of the recipient animal. Thus the peripheral paralysis of the respiratory muscles might be distinguished from central paralysis. Dogs under morphine and urethane anesthesia were used. Chlorazol fast pink was used as anticoagulant. The recipient dog's head was connected to its body by means of the vertebral column, the vagi and inferior laryngeal nerves. Division of the neck was made below the larynx and above the origin of the phrenics. The vertebral arteries and veins were tied off. The respiration of both animals were recorded by tambours connected to pneumographs about the chest and in later experiments to intra-laryngeal balloons also. The femoral arterial blood pressure of both animals was recorded. An intact phrenic of each animal was prepared for stimulation.

In one instance, when 100 mgm. of 10 per cent alkaloidal nicotin in Lockes was injected into the carotid artery to recipients head, a central paralysis resulted, whether spinal or medullary, was not determined. In-

jection of lethal doses of nicotin into the donor's femoral vein plus artificial respiration to donor did not paralyse the recipients respiration. Injection of lethal doses of nicotin into the femoral vein of donor without artificial respiration indicated that the peripheral paralysis developed in the donor before the respiratory center failed in the recipient. The respiratory center of the recipient would of course fail from asphyxia after the donor has ceased to breathe because of a peripheral paralysis.

Nicotin injected into the body of the recipient quickly resulted in paralysis of the diaphragm and chest while the center was still active as shown by the laryngeal record.

*An ovarian estrogen with effects limited to the vagina.* S. C. FREED (by invitation), S. D. MESIROW (by invitation) and SAMUEL SOSKIN. Department of Metabolism and Endocrinology, Michael Reese Hospital, Chicago, Illinois. (Read by title.)

The composite nature of the estrus phenomenon has been demonstrated in several previous communications. We have observed cyclic vaginal cornification associated with an infantile uterus, in adult female rats which had been subjected to x-ray irradiation at 21 days of age. We have also shown that these results were not due to the effects of the irradiation on the uterus, since this organ was still capable of responding to injections of dihydroxyestrin benzoate. It remained to rule out the possibility that the site of action of the x-rays might have been on the gonadotropic mechanisms rather than on the ovary itself.

Twenty-one day old rats were irradiated with 2 doses of 450 Roentgen units, one week apart. At 40 days of age they were injected with APL, and examined for their estrus response. In all cases the vagina was open and the mucosa cornified. The uteri, however, remained more or less infantile averaging 54 mgm. in weight. Normal rats, after similar treatment with APL had uteri averaging 180 mgm. in weight. Microscopically, the uterine epithelium of the irradiated rats ranged from the completely infantile state to that of moderate proliferation, but never approached that of the normal injected rats nor of irradiated rats treated with dihydroxyestrin. The ovaries, though small, contained large follicles and corpora lutea.

Since it has been previously shown that the uterus is not at fault, the almost exclusively vaginal effects of the APL administration must be ascribed to a limited response of the irradiated ovaries. For a number of reasons, we do not believe that the above results can be explained as a threshold phenomenon, based on the diminished secretion of a single estrogen. It is concluded that the irradiated ovaries secrete an estrogen, the effects of which are more or less limited to the vagina.

*Weight loss by insensible perspiration, skin temperatures and oxygen consumption rates under various environmental conditions and in relation to each other.* HARRY FREEMAN (introduced by R. G. Hoskins). Research Service, Worcester State Hospital, Worcester, Mass. (Demonstration.)

Studies have been made of the total insensible perspiration loss and its components, the skin and pulmonary loss of weight, under conditions in which the body loses heat (24°C. and 20 per cent relative humidity), and where the body gains heat (39°C., 20 per cent relative humidity).

At a neutral condition (30°C.), the rate of total insensible perspiration loss, the skin temperature, and the oxygen consumption rate have been determined under conditions of basality, non-basality, and where the metabolic level has been increased by the administration of dinitrophenol, and also glycine. The levels of the individual factors and their relationship to one another are shown by graphs and tables.

*Relation of the cervical sympathetics to pseudopregnancy in the rat.* H. B. FRIEDGOOD and S. BEVIN (by invitation). Harvard Medical School, Boston, Mass.

The regularity of the normal rat's estrous cycle can be interrupted by a prolonged period of diestrus (pseudopregnancy) as a result of glass rod or electrical stimulation of the cervix uteri. These procedures probably result in pseudopregnancy by dispatching a neural stimulus to the anterior hypophyseal gonadotropic cells. In searching for a likely pathway for this neural impulse, the cervical sympathetic nerves have been studied because there is evidence that they innervate the anterior hypophysis. Without anesthesia the cervix was stimulated in normal and operated animals. The glass rod was inserted into the vagina and repeatedly brought into contact with the cervix. For its electrical stimulation, the cervix was exposed with a speculum, and condenser discharges were applied through bipolar, silver-wire electrodes at the rate of 60 per second.

| | NUMBER OF RATS | EXPERIMENTAL METHOD | NUMBER OF RATS DEVELOPING PSEUDOPREGNANCY | NUMBER OF RATS WITH UNAFFECTED ESTROUS CYCLES |
|---|---|---|---|---|
| Control | 10 | Electrical stimulation of the cervix uteri | 10 (100)* | 0 |
| | 31 | Glass rod stimulation of cervix uteri | 28 (84) | 5 (16)* |
| Experimental | 34 | Electrical stimulation of cervix uteri after cervical sympathectomy or superior ganglionectomy | 19 (55.8) | 15 (44.2) |
| | 23 | Glass rod stimulation of cervix uteri after cervical sympathectomy or superior ganglionectomy | 15 (65.2) | 8 (34.8) |

* Figures in parentheses indicate percentages.

Cervical sympathectomy or superior cervical sympathetic ganglionectomy interferes materially with the incidence of pseudopregnancy after glass rod or electrical stimulation of the cervix uteri. Since mating with a vasectomized male almost invariably produces pseudopregnancy in a sympathectomized female, there must be another and probably more important neural pathway to the anterior hypophysis. Although the gonadotropic activity of the anterior hypophysis may be modified experimentally by exclusion of the cervical sympathetics, one cannot conclude that they normally participate in stimulation of the pituitary.

*The clearance of vitamin C by the human kidney.* GERALD J. FRIEDMAN (by invitation), CHARLES MCGOEY (by invitation) and ELAINE P. RALLI. Laboratories of the Department of Medicine, New York University College of Medicine, New York City.

Vitamin C clearances have been done on normal and scorbutic subjects

both with and without simultaneous inulin clearances. The procedure described by Smith, Goldring and Chassis was followed in determining the clearances. The 2–6 dichlorophenolindophenol titration method was used for the determination of vitamin C.

The reabsorption of vitamin C was practically complete at plasma levels below 2 mgm. per cent; as the plasma level was raised above this value, the vitamin C—inulin clearance ratio rises abruptly and approaches 1.0 as the limiting value.

Vitamin C clearances without simultaneous inulin clearances were done on a scorbutic patient whose plasma concentration prior to the administration of vitamin C was 0.36 mgm. per cent. In the first experiment this low concentration was maintained by giving a saline infusion with no vitamin C. The clearances in this experiment were constant at 4 cc. per minute. The same patient was then studied at plasma concentrations varying from 5 to 22 mgm. per cent. Between these plasma concentrations the clearance of vitamin C was constant at 114 cc. per minute. These results suggest that vitamin C is probably a threshold substance in the sense of being reabsorbed from the glomerular filtrate when the plasma concentration is small.

*The influence of environmental factors on the rate of restitution of the gonadotropic factor in the discharged rabbit pituitary.* MAURICE H. FRIEDMAN and GERTRUDE S. FRIEDMAN (by invitation). Department of Physiology, University of Pennsylvania, Philadelphia.

In order to study the influence of dietary protein on the rate of synthesis and storage of the gonadotropic factor, use was made of R. T. Hill's finding that the act of coitus discharges the female rabbit pituitary almost completely within 24 hours. Confirming Hill, we have found that the rate of restitution of the gland gonadotropic content follows a characteristic curve which returns to the oestrous level (pre-discharge) by the 10th day following coitus. An appraisal of the influence of dietary protein was made difficult because of a gradual shift in the *level* of the curve over a period of months. On all diets used the values obtained in the spring were consistently higher than those obtained in the fall and winter. Inspection of the data gathered over a period exceeding two years gives the impression of a true seasonal variation. Nevertheless, it has been impossible to increase the autumn values by exposure of the rabbits to artificial light for as long as 50 days (8 hours added light nightly), or to decrease the spring values by subjecting rabbits to complete darkness for as long as 30 days. In view of our inability to fix the level of the curves by control of the light ration it is necessary to repeat the experiments on various deficient diets with strictly parallel controls of animals on complete diets.

*The influence of intravenous injection of a colon bacillus vaccine on the motility of the dog's colon.* E. A. GALAPEAUX (by invitation), HARRY F. ADLER (by invitation), R. L. FERGUSON (by invitation) and R. D. TEMPLETON. Departments of Physiology and Pathology-Bacteriology of Loyola University, School of Medicine, and the Department of Physiology of The University of Chicago.

In this study we used 3 cecostomized animals which had previously

been trained to lie quietly for the time required in the experimental procedure. The recording technique and the method of computing activity was the same as that described in a previous paper (Proc. of this Society, 1937).

The vaccine used was prepared from a 48 hour nutrient agar culture of E. coli (communior of laboratory stock). The growth was washed from the culture plates with a small quantity of sterile tap water into vaccine bottles and subjected to 56°C. in a water bath for 30 minutes. A test to insure sterility and a count to approximate concentration was made. Before injection, this stock vaccine was diluted to contain approximately 650,000 per cubic millimeter.

Prior to the injections 30 control tracings of 400 minutes each were obtained. A computation of the motility in each 50 minute period revealed the activity to be well above 50 per cent throughout the experiment.

In 12 experiments 1 cc. of the diluted vaccine was injected after a 200 minute control period. The general effect was a depression of motility to below 50 per cent beginning in the first 50 minutes and becoming most pronounced in the last period, i.e., between 150 and 200 minutes after the injection.

All of the animals responded with considerable retching between 50 and 150 minutes after the injection. Two of the 3 animals used invariably vomited and defecated at about this time. The activity was invariably greater immediately prior to defecation and during the first part of the act, than it was a few minutes before or after the act.

Four experiments were continued for 1000 minutes after injecting the vaccine. The activity continued below that of the controls throughout the period studied. The marked depression which commonly occurs at about 150 minutes lasted for approximately 400 minutes after which the activity gradually returned.

*Extension of a conflict based upon food to other physiological systems and its reciprocal relations with sexual functions.* W. Horsley Gantt. Pavlovian Laboratory, Phipps Clinic, Johns Hopkins University, Baltimore, Md.

A chronic, obstinate nervous disturbance was produced in dogs by difficult differentiations of signals for food. The permanence of such a neurosis depends upon constitution. In one dog the condition, resembling an anxiety state, has lasted without intermission for five years. The chronicity afforded opportunity for a study of the total behavior. An extension to involve other systems than the food (respiratory, sexual) with generalised defense occurred gradually. Even after four years' rest without intervening practise a repetition of the original food signal or of the environment produces a stereotyped symptom-complex characterised by defense reactions—negativism, refusal of food, whining, barking, agitated movements, increased respiration, raucous forced breathing, frequent uncontrolled micturition, sexual erections. A special investigation was made of the reciprocal relations between this "neurosis" originating with food and another fundamental function (sex). Besides the manifestation of spontaneous sexual erections in the experimental camera, the neurosis had a marked and constant effect upon the latent period, duration and type of induced erections and ejaculation; a condition resembling pre-

mature ejaculation resulted. Outside the experimental camera these sexual functions were normal. The camera and the former conditioned food signals had a specific effect upon the sexual reflexes in the neurotic dog. Control dogs who also had worked in the camera but without conflict showed no difference in the above sexual reflexes when they were placed (a) inside and (b) outside the camera. Conversely, sexual stimulation (a bitch in estrus) markedly affected the defense reactions resulting from the food conflict, temporarily abolishing them. Human social factors (petting during the conditioned signals which formerly produced food responses but now defense reactions) also inhibited the expression of anxiety. Thus, not only does a conflict arising in one physiological system (food) involve many other systems but there is evidence of reciprocal influences between the systems. In other words there is a tendency toward disintegrative action as a psychobiological unit.

*The nervous secretion of saliva: the relation of the conditioned reflex to the intensity of the unconditioned stimulus.* W. HORSLEY GANTT. Pavlovian Laboratory, Phipps Clinic, Johns Hopkins University. (Read by title.)

Previous studies from this laboratory show that the unconditioned parotid secretion is a linear function (Secretion = a + bQ, where a and b are constants for each dog, and Q = amount food). The conditioned reflex also bears a relation to amount of food, increasing as the food increases, but the relation is of a much more complex character. The conditioned reflex is dependent upon the nature and intensity of the conditioned signal as well as upon the amount of food. While with the unconditioned reflex this quantitative adaptation is immediate, automatic and independent of the dog (except for weight and certain physical factors), the adaptation of the conditioned reflex is accomplished only after considerable practise, is dependent upon the individual. The formula for the intensity of conditioned reflex is, $Cr = a + b(1 - e^{cQ})$; this exponential formula for the conditioned reflex thus differs from the linear formula for the unconditioned reflex; it is suggestive of the Weber-Fechner Law, while the unconditioned reflex formula is similar to those for gastric and pancreatic secretions.

Furthermore the adaptation through the conditioned signal to the amount of food imposes a burden upon the nervous system. Although the adaptation on the unconditioned reflex level is performed equally well by all dogs, animals with a weak nervous system cannot elaborate and maintain stably differentiated conditioned reflexes on the basis of the amount of food accompanying each. However with capable and strong animals—classified both by their general behavior and their behavior under stress—there is elaborated a stable adaptation in both the motor and secretory conditioned reflexes including latent period as well as intensity of the conditioned reflex.

These experiments show that the intensity of conditioned reflex depends not only upon 1, the nature of the signal (whether visual, auditory, olfactory etc.); 2, the intensity of the signal, (Kupalov, Lyman) but also 3, a logarithmic relation to the unconditioned stimulus.

*Posterior nerve function as tested by the conditioned reflex method.* W. HORSLEY GANTT and ROGER BROWN LOUCKS (by invitation). Pav.

lovian Laboratory, Phipps Clinic, Johns Hopkins University. (Read by title.)

Some doubt has been thrown recently by Foerster on the Bell-Magendie Law regarding the conduction of pain impulses; it has been claimed that certain of these travel through the anterior nerve roots. The conditioned reflex method provides an adequate technique for testing this law in animals. Conditioned reflexes were formed by applying a tactile stimulus on the two opposite sides of the body, and afterwards the posterior nerve roots supplying that area on one side of the body were cut inside of the spinal canal. The measure of the conditioned reflex was the secretion of saliva to the tactile stimulus, which was made a signal for food. Posterior nerve roots were cut both in the thoracic and in the lumbar and sacral regions. Due to overlapping, more than five roots had to be severed in order to provide an area of sufficient size (about 2 cm. diameter) which was completely deprived of tactile fibers, judging by the ability to form a conditioned reflex to the tactile stimulation of this area. A leg was rendered apparently completely anesthetic to tactile stimuli by cutting the 2nd, 3rd, 4th, 5th lumbar and 1st, 2nd, sacral posterior roots. However pain reactions to the faradic current could still be obtained from the foot, anterior and posterior borders of the whole leg, and also conditioned reflexes could be formed on the basis of the pain stimuli at these points. The conclusion is that both pain and tactile reception from the skin is abolished by cutting the posterior nerve roots, but that there is considerably more overlapping for the pain fibers than there is for the tactile.

*Reflex excitation and inhibition of sympathetic activity.* J. B. Gaylor and O. V. Schwerin (introduced by D. W. Bronk). Johnson Foundation, University of Pennsylvania, Philadelphia.

Previous work has shown that the spontaneous activity of the sympathetic centers as indicated by action potentials in the inferior cardiac nerve can be inhibited by stimulating the central end of the vagus or carotid sinus nerves at frequencies above 15 per second. If the stimulus frequency be 2 to 5 per second, grouped discharges of impulses occur in rhythm with the stimuli. The question arises as to how stimulation of afferent fibers at one frequency can produce rhythmic activity and complete inhibition at another. This has been one object of our work. We have also considered the possible relation of the frequency at which sympathetic centers can be driven to their natural frequency of discharge.

We find that both inhibition and driving of the centers can be produced by stimulation of various other nerves such as the aortic, femoral, and phrenic, and by stimulation of the cerebral cortex. We therefore conclude that the phenomena under consideration are not due to the effects of impulses from one specific set of receptors but is a general result of afferent impulses and the properties of the centers. In agreement with this assumption is the observation that during inhibition produced by any one means, such as distension of the lungs, it is difficult to drive the centers by periodic excitation of another nerve.

These facts, and others, suggest that a volley of afferent impulses produces less inhibition than excitation, but that the inhibitory state persists longer than the excitatory. At low frequencies each volley would there-

fore excite the centers. At higher frequencies there would be summation of inhibition to a degree sufficient to overcome the excitation and to suppress the activity of the centers. Long persistence of inhibition after high frequency stimulation and the relative effects of different strengths of stimulation agree with this tentative hypothesis.

The frequencies at which one can drive the discharge rhythmically are about the same as those of volleys discharged from the centers in normal activity. This appears then to correspond to some characteristic time constant of these cells.

*Studies on the effect of increased intracranial pressure.* ERNST GELLHORN and LOUIS YESINICK (by invitation). Department of Physiology, College of Medicine, University of Illinois, Chicago. (Read by title.)

In order to decide whether the circulatory disturbance (asphyxia) or the mechanical stimulation of the brain is responsible for the circulatory adjustment (rise in blood pressure) which follows an increase in intracranial pressure (i. p.) to the blood pressure level, numerous experiments were carried out in dogs under amytal, chloralose and chloralosane anesthesia and artificial respiration with and without double vagotomy. It was found (1) that the bl. pr. reaction to increased i.p. is greatly increased during a period of oxygen deficiency induced by inhalation of 7 per cent $O_2$; (2) that the determining factor for this reaction is not the absolute level of the i.p. but its relation to the bl. pr. Further experiments deal with the effect of insulin hypoglycemia on Cushing's reaction. It is shown that at a low blood sugar level the rise in bl. pr. accompanying a rise in i. p. is greatly increased. The reaction is similar to that observed during inhalation of low oxygen. The experiments support the hypothesis that the effect of hypoglycemia on the central nervous system is essentially that of anoxia (cf. Moldavsky and Gellhorn, Proc. Soc. Exper. Biol. **36:** 92, 1937; Ingraham, Moldavsky and Gellhorn, Am. J. Physiol. **119:** 341, 1937; Glickman and Gellhorn, Am. J. Physiol. **121:** 358, 1938).

*An apparatus for the determination of the respiratory metabolism of tissue cultures.* C. L. GEMMILL and G. O. GEY (by invitation). Johns Hopkins School of Medicine, Baltimore, Md. (Demonstration.)

The Dixon-Keilin manometric apparatus has been modified for the determination of the respiratory activity of tissue cultures of normal and malignant cells grown by means of the roller tube technique. From one to two hundred colonies of pure strains of cells are cultivated on the inner wall of a cylinder which is inserted into a roller culture tube. After a suitable period of growth, the capsule is transferred from the roller tube to the apparatus. A glass pump, operated by an external magnet, circulates the fluid from the lower part of the vessel to the upper chamber where there is adequate space for transfer of gas. This method for studying tissue metabolism has the advantage of using pure strains of cells grown under controlled external conditions.

*Synaptic transmission.* ROBERT GESELL and JACK LAPIDES (by invitation). Department of Physiology, University of Michigan, Ann Arbor. (Read by title.)

Routine electrical stimulation of two afferent channels of similar function is accepted to be more effective than stimulation of either path

alone. Separate and combined faradic stimulation of the central ends of the vagal nerves with symmetrical rectangular shocks however showed divergent results. When both nerves were simultaneously stimulated with the shocks in phase, the individual inhibitory effects were annulled. When the shocks were 100 per cent out of phase, they were summed. (Robert Gesell and Jack Lapides, University of Michigan Hospital Bulletin, January, 1938, p. 1.) The annulment is now shown to be artifact.

*An apparatus for the study of the effects of gamma rays of radium on pure strain cultures of normal and malignant cells.* GEORGE O. GEY (introduced by Carl H. Hartman). Johns Hopkins University, School of Medicine. (Demonstration.)

For the study of the biological effects of the gamma rays of radium on living normal and malignant cells a special radiation chamber was constructed which permits a simultaneous observation of the effects of large and small doses on tissue cultures maintained in roller tubes. The position of the culture tubes in the chamber allows a variation in intensity from 1.0–2,000 with many intermediate positions. The apparatus is to be used for a study of the possible mutagenic properties of gamma rays following prolonged exposures to sublethal doses, and for a study of the effects of gamma rays on the metabolism and growth of cells maintained in roller tube cultures.

*The character of the chemical oscillators responsible for the electro-encephalogram.* FREDERIC A. GIBBS. Department of Neurology, Harvard University, Cambridge, Mass.

Studies on the electrical activity of the cerebral cortex indicate that it, and probably the whole brain, is an aggregation of small chemical oscillators which can join together to form larger oscillators. The nature of these chemical oscillators is suggested by what is known of the structure, chemistry and metabolism of the brain, and by the alterations which can be produced under normal and abnormal conditions in the electrical activity of the cortex. Especially significant in this connection is a recent investigation (with E. L. Gibbs, Williams and Lennox as collaborators) of the relation between the frequency of cortical potentials and the supply of sugar, $O_2$ and $CO_2$ to the brain.

The characteristics of a chemical oscillator can be described in terms of the chemical equivalents of energy, tension and mass, to use the terms applied to mechanical oscillators. The energy supply in the case of brain oscillators appears to be derived from the oxidation of sugar. $CO_2$ can be regarded as an equivalent of tension: the size and permeability of the cell or functional unit as mass.

A model of this general type of chemical oscillator is provided by a burning candle in a partially closed container. A number of such oscillators interconnected and placed in a larger partially closed container will show properties which are characteristic of the electrical activity of the cortex.

*The osmotic work of the kidney following the injection of hypertonic NaCl, urea, and their combination.* ALFRED GILMAN and NANCY E. KIDD (by invitation). Laboratory of Pharmacology and Toxicology, Yale University School of Medicine, New Haven, Conn. (Read by title.)

Intravenous injections of hypertonic NaCl, urea, or their combinations were made in trained unanesthetized dogs. Urine was constantly collected for 180 minutes by means of a retention catheter in the bladder and analyzed for NaCl, urea, and total osmotic pressure. The total osmotic pressure of the blood was also followed. Following the injection of all concentrations of NaCl from 1.5 per cent to 24.0 per cent the highest NaCl concentraion obtained in the urine (osmotic ceiling) was constant at approximately 1.8 per cent and little influenced by rate of flow. Following the injection of hypertonic urea solutions isosmolar to the NaCl employed (3.0 per cent–48.0 per cent) the concentration of urea in the urine varied with the concentration in the blood and the rate of urine flow and reached an osmotic ceiling (8.0 per cent) over twice as high as that observed for NaCl. When combinations of hypertonic NaCl and urea were administered, the presence of high concentrations of urea in the urine had absolutely no effect on the osmotic ceiling of NaCl.

If the loop of Henle is the site of osmotic work of the kidney, and urea fails to influence the degree to which NaCl can be concentrated at this site, then urea is either absent from the loop of Henle or else exerts no osmotic effect there. The first explanation tacitly assumes complete reabsorption of urea in the proximal tubule and subsequent secretion by the distal tubule. In support of the second explanation is the concept of the partial back-diffusion of urea. If such back-diffusion takes place in the loop of Henle it is conceivable that the epithelial cells of this portion of the kidney attain a urea concentration equal to the fluid in the tubules and thus no osmotic gradient referable to urea exists. More evidence is being obtained on these points.

An interesting observation is that the mercurial diuretics even when increasing diuresis rate by 300 per cent do not lower the osmotic ceiling for NaCl.

*Changes in accommodation in heart muscle following a previous response.* A. S. Gilson, Jr. Washington University School of Medicine, St. Louis, Mo.

Reports upon determinations of refractory periods in heart muscle have generally failed to consider the effects of the form of the stimulating current upon the measured thresholds. We have studied the changes in excitability in heart muscle following a response. In addition to stimuli of the usual forms, we have used S-shaped currents which rose slowly and at a controllable rate to a plateau which could be terminated at any desired time. Such stimuli permit recognition of changes in accommodation which are not seen if only short shocks are employed. Qualitatively in agreement with the observations of Bonnardel (Arch. Int. de Physiol. **43:** 107, 1936) on toad hearts, it was found that freshly prepared and unperfused turtle ventricular strips and non-rhythmic turtle ventricles perfused with buffered, oxygenated saline revealed slow accommodation. Nevertheless, observations made at various times following an induced response showed a long-lasting period during which there was a marked elevation to slowly rising currents (0.05 sec. or longer to 90 per cent of plateau level) although the threshold to a short shock (RC less than about 0.004) might be nearly or quite normal. With strips, it was possible to observe these effects for at least a half minute following the previous response. The phenomenon is not attributable merely to after-effects of

the stimulating current inducing the previous response and may be seen, for example, in a strip preparation stimulated near the middle by a condenser shock and then tested by a slowly rising current applied through electrodes at the two ends. It is, for example, enhanced by fatigue or a perfusate not oxygenated, unbuffered, or with increased potassium content.

It is concluded that during the recovery of heart muscle from a previous response one may observe an increase in the accommodation developed by a succeeding slowly rising current for a time which greatly exceeds the period of relative refractoriness revealed by a short shock.

*After-potentials of polarized nerve.* HELEN TREDWAY GRAHAM. Department of Pharmacology, Washington University, School of Medicine, St. Louis, Mo.

The negative after-potential of a single nerve response (A fibres of frog sciatic or cat saphenous), like the spike potential which it resembles in electrical sign, is increased at the anode and decreased at the cathode of a polarizing current. For a given low degree of anodal polarization, the increase of negative after-potential is relatively very much greater than that of the spike; for instance, polarization that increases the maximum spike height by 25 per cent multiplies the height of the negative after-potential several times, and affects its duration similarly. Cathodal polarization that reduces the spike height about 25 per cent cuts down the negative after-potential of frog nerve response to less than half its original size, and removes all the after-negativity of cat nerve response. The effects on the after-potential increase with the strength of the polarizing voltage, but are relatively greater when the voltage is small.

The positive after-potential of the response of cat nerve is increased at the cathode and decreased at the anode of a polarizing current. The cathodal increase of positive after-potential is much less marked than the anodal increase of negative after-potential, and is accompanied by decreased instead of increased duration. Anodal polarization, even when very weak, suppresses all positive after-potential at the normal interval after the shock; but the greatly increased negative after-potential manifested during stronger anodal polarization (enough to produce about 25 per cent increase of spike height) is followed, in the response of cat or frog nerve, by a positive after-potential falling later than the positive after-potential in the response of unpolarized nerve.

The after-potential level obtaining during a rhythmic series of responses is affected by polarization as would be predicted from the effects of polarization on the after-potentials of single responses, and may be changed in sign as well as height by suitable polarization.

*Studies on the Vulpian-Heidenhain phenomenon.* R. P. GRANT and W. M. M. KIRBY (introduced by J. C. Hinsey). Department of Physiology, Cornell University Medical College, New York, N. Y. (Read by title.)

In cats, the 12th (hypoglossal) nerve was sectioned on one side. After 7 days more the animals were anesthetized with nembutal, the opposite normal 12th nerve was cut, and the animals prepared for recording contractures in the tongue. The best degeneration time was found to be from 7 to 10 days. Stimulation of the cervical sympathetic trunk low

in the neck produced a typical contracture in nine animals. After removal of the superior cervical ganglion and the cervical sympathetic trunk, as well as section of the 12th nerve (five animals), stimulation of the chorda-lingual nerve caused contracture. Likewise, after complete removal of the cervical sympathetic trunk from the superior cervical to the 2 T or 3 T chain ganglia and section of the 12th nerve (five animals), the contracture was produced by appropriate stimulation of the chorda tympani and the chorda lingual nerves. It is concluded that in the cat there are components in both the chorda tympani and the cervical sympathetic trunk which produce pseudomotor responses in the tongue.

In two dogs, the vagus was cut below the nodose ganglion and the 12th nerve close to its exit from the cranium. 8 and 11 days later, stimulation of the vago-sympathetic trunk and the superior cervical ganglion in these dogs failed to produce a contracture, while contractures were present on stimulation of the chorda lingual. In one of these, the response was elicited very well on stimulation of the chorda tympani. In two other dogs, the 12th nerve was sectioned, and 10 days later the stellate ganglion when stimulated failed to elicit a contracture, while it was possible to attain contractures from the chorda lingual. These experiments indicate that in the dog stimulation of the cervical sympathetic does not produce the contracture.

*The Fourier transform of the electro-encephalogram.* ALBERT M. GRASS (by invitation) and FREDERIC A. GIBBS. Department of Neurology, Harvard University, Cambridge, Mass.

A differential analyzer has been developed to apply the Fourier transform to an arbitrary amplitude vs. time function, such as an electroencephalogram. The final result is a continuous plot of the amplitude vs. frequency function. A representative oscillogram is taken as a shadowgraph and later made a continuous function by forming an endless belt. It is then passed between a light source and a photo-cell. The resulting signal is passed through an extremely sharp variable electric wave filter. The amplitude of each differential frequency component is measured and plotted automatically. No claim is made here that the actual frequencies plotted originate as pure sine waves, such as the Fourier transform would indicate if misinterpreted. We are only presenting a method of analyzing brain waves in a mathematical way which will allow a common and rigorous expression. This method is independent of the several intricate and arbitrary terminologies which have been devised for compiling results and which have lead to conflicting interpretation of identical findings. This method is also a tremendous time saver as well as a guesswork eliminator, as it ends the tedious work of measuring frequency by hand and of estimating for each component the percentage of time present. This latter, if done as accurately, would take an infinite amount of time for a single record. However, the instrument is meant not to substitute but rather to supplement oscillographic recording technique used at present, since the latter is indispensable for the indication of short bursts of particular wave forms, phase relations and "build ups" which have been shown valuable and which are lost in the single analyzed record, since it eliminates the time factor. Nevertheless, this time element can be supplied in the form of different analyses made at separate or continuous intervals of time.

With this technique, we have determined quantitatively the difference between the activity of different parts of the head in the same subject, between the same parts of the head in different subjects, and the same parts of the head of the same subject at different times. We have compared quantitatively the records of normal individuals with those of patients suffering from various cerebral disorders, which is a step toward determining the range of normal and the establishment of absolute criterior for differential diagnosis.

*Corticifugal pathways mediating vasomotor impulses.* HAROLD D. GREEN and HOMER HARTZELL (by invitation). Department of Physiology, Western Reserve University, School of Medicine, Cleveland, O.

In previous publications one of us in collaboration with E. C. Hoff demonstrated that electrical excitation of nervous elements within the cerebral cortex initiates vasomotor activity. The present study has been undertaken to determine the corticifugal pathway involved.

Cats were anesthetized with ether, curarized and given artificial respiration. The cerebrum was sectioned coronally, the sections sloping caudally, and the cut surface stimulated by a stigmatic electrode with 60 cycle current, strength 0.5 to 2.0 volts while recording blood pressure. The reactive points were determined by gross inspection of the cut surface. Sections were made about every 3 mm. extending from the frontal pole of the cortex to the third nerve in the different animals.

Successful experiments were completed in 12 cats. A rise of pressure of from 10 to 50 mm. Hg, similar to that obtained from excitation of the cortex, could be elicited from points in the white matter along rather sharply delimited pathways from the gyrus prorius and the anterior and posterior sigmoid gyri passing superiorly and inferiorly to the posterior extension of the cruciate sulcus and converging laterally to the lateral ventrical and head of the caudate nucleus to enter the ventral portion of the internal capsule. Similar responses were obtained from the hypothalamic region. Posterior to the hypothalamus exact location by gross inspection was difficult, the responses were obtained from a region extending 3–4 mm. laterally from the midline and from the aqueduct ventrally almost to the surface of the brain stem. Definite responses were not obtained from the cerebral peduncles.

These results appear in harmony with those of Kabat, Ranson and Magoun (Arch. Neurol. and Psych. **34:** 931, 1935) regarding the pathways caudal to the hypothalamus. In addition they demonstrate the presence of projection fibers from the cortex to the hypothalamic level which were not described by the above authors. Their failure to observe these descending vasomotor pathways may have been due to their choice of anesthetic (see Green and Hoff, Am. J. Physiol. **118:** 641, 1937).

*Preliminary notes on the nerve control of the coronaries of the Macacus monkey.* CHARLES W. GREENE. University of Missouri, Columbia. (Read by title.)

The arrangement of the blood vascular system in the heart of the Macacus monkey is quite inconstant in plan, especially as to the drainage of the venous system. The coronary sinus, which is so prominent in the dog, is less significant, and receives fewer coronary veins. The azygos vein is more prominent and certain coronary veins enter that channel to

the right auricle. It is more difficult, therefore, to cannulize the coronary venous system in making the measurements on which our investigations rely for evidence of nerve control of coronary volume of flow.

However, the experiments to date point to a well-developed coronary vasodilator system, in which the efferent nerve fibers reach the coronaries chiefly by way of the inferior cervical ganglion, and the sympathetic branches that contribute to the cardiac plexus. Such dilator drugs as adrenalin act on this system to produce coronary dilation, together with cardiac acceleration. Thus far no evidence of dilator pathways by way of the superior cervical ganglion have been found. A number of drugs have had preliminary testing, but the duplicates do not yet permit of generalization.

The arrangement of autonomic sympathetic nerves in the region of the annulus and the inferior cervical ganglion is much more distinctly separated from the cranial vagus complex than in the dog, and seems to correspond more with the anatomical pattern of the human.

*Can expiratory inflation occur during hyperpnea produced by physical exertion?* JAMES A. GREENE, L. W. SWANSON and R. H. HEEREN (introduced by F. M. Smith). Department of Internal Medicine, State University of Iowa, College of Medicine, Iowa City.

In a previous study plethysmograms showed that expiratory inflation occurred during hyperpnea produced by physical exertion. These results have been questioned. It is for this reason that the increase in volume of tidal air during hyperpnea produced by physical exertion was compared with the volume of the resting complemental air in order to ascertain whether or not expiratory inflation can occur. The measurements were made with the subjects in the supine position. There were 29 observations on 9 subjects.

The results show that expiratory inflation could have occurred in 27 instances. In 2 instances the physical exertion was carried to an extreme, and under such conditions the results show that expiratory inflation very likely could not have occurred.

*The experimental production of intersexuality in the rat.* R. R. GREENE and M. W. BURRILL (introduced by A. C. Ivy). Northwestern University Medical School, Chicago.

Large amounts of androgenic substance have been administered to pregnant rats. The genetic female offspring have been permanently masculinized. The degree of masculinization seems to depend on the amount given and also on the period of pregnancy when treatment is administered. Seventy-six very definitely intersexed rats have now been produced and examined.

Well affected animals have a penis, Cowper's glands and the various prostatic lobes. The lower vagina is absent. In some animals the dilated upper vagina ends blindly. In others it empties into the urethra by bilateral ostia medial to the Wolffian ducts. Animals have been produced with head and tail of the epididymis connected by efferent tubules to the rete of the gonad and continuous with the vas deferens which parallels the uterus and empties into the urethra. Evidences of Mullerian duct inhibition have been slight.

The gonads in well affected animals are also masculinized. They are

very small with a thick tunica albuginea and no corpora lutea. The rete is extensive and medullary tubules extending into the stroma of the gonad have been found.

*The experimental production of intersexuality in the rat.* R. R. GREENE (by invitation), M. W. BURRILL (by invitation) and A. C. IVY. Northwestern University Medical School, Chicago. (Demonstration.)

Wax plate reconstructions of the genital apparatus of the normal male and female rat, and of intersexed rats, which have been produced by injecting the pregnant rat with testosterone, will be demonstrated. In addition, whole mounted specimens, drawing and microphotographs of the organs of the intersexed animals will be displayed.

*The spectral absorption of sulfanilamide and its red azo dye.*[1] MAGNUS I. GREGERSEN and E. E. PAINTER (by invitation). University of Maryland School of Medicine and College of Physicians and Surgeons, Columbia University.

Spectrophotometric determination of the red azo dye prepared according to Marshall's procedure for the analysis of sulfanilamide increases the accuracy of the analysis, eliminates the routine preparation of azo dye standards, and reduces the size of the sample required. The peak of the spectral absorption curve of this red azo dye lies at 530m$\mu$. Analysis of standard solutions of sulfanilamide prepared in either water or serum (proteins precipitated with trichloracetic acid or p-toluene-sulfonic acid) give the same optical density, the latter being directly proportional to the concentration of sulfanilamide within the range investigated (0–10 mgm. per cent). With 0.5 cc. samples the error in analysis never exceeded 1.5 per cent.

In attempts to make simultaneous measurements of 1, plasma volume with T-1824; 2, extracellular fluid volume with NaSCN, and 3, total body water with sulfanilamide, we observed that thiocyanate interferes with the coupling of dimethyl $\alpha$-napthylamine with sulfanilamide. Seeking to eliminate this chemical procedure we investigated the possibility of direct spectrographic analysis in the ultraviolet, where, according to its structural formula, sulfanilamide should have a definite absorption spectrum. The preliminary experiments (Hilger quartz spectrograph equipped with Spekker photometer and hydrogen discharge tube, loaned by Dr. H. T. Clarke) revealed a single broad band in the region 230–290m$\mu$, with maximal absorption at 259.5m$\mu$. At this wavelength the extinction coefficient of a 1 mgm. per cent solution (aqueous) is 0.9. Spectrographic analysis for sulfanilamide in blood may be carried out satisfactorily on the alcoholic extract.

*The immediate effects of coronary sinus occlusion on the dynamics of coronary blood flow.* DONALD E. GREGG and DONALD DEWALD (by invitation). Western Reserve University, School of Medicine, Cleveland, O. (Read by title.)

Following coronary sinus ligation in anesthetized dogs, the systolic and diastolic inflow into the left coronary (determined by differential pressure

[1] Aided by grants (to M. I. Gregersen) from the Committee on Grants-in-Aid, National Research Council and The Rockefeller Foundation.

curves) is greatly reduced. The reason is that, although the contour and time relations of the peripheral coronary pressure curve are similar to those under normal conditions, both the diastolic and systolic resistances are elevated. In fact the latter (when raised to its proper ordinate value) equals the aortic systolic pressure. The intra-sinus pulse assumes the general pattern of the peripheral coronary pressure curve except that the systolic values range between pressures less than to those occasionally greater than aortic systolic.

In view of these pressure relations and since the coronary sinus drains a goodly share of the left coronary it is possible that sinus occlusion prior to occlusion of a left coronary ramus might establish a peripheral blood flow adequate to maintain contraction in a potentially infarcted area.

In the same experiments when sinus occlusion was added to central coronary clamping was found that 1, the coronary back flow increased from a maximum of about 1 cc./min. of arterial blood to a maximum of 37 cc./min. of blood containing only 1 to 3 volumes per cent oxygen, and 2, the source of this extra blood was largely the other non-occluded coronary arteries. However this additional blood flow was not sufficient to prevent failure of contraction (determined by myographic records) and hence was of no material benefit to the myocardium.

*Dynamics of collateral circulation following chronic occlusion of coronary arteries.* DONALD E. GREGG and FREDERICK R. MAUTZ (by invitation). Departments of Physiology and Surgery, Western Reserve University, Cleveland, O.

In different dogs either the right coronary, the left circumflex or the left descendens was occluded in multiple stages. Two to twelve months after such complete occlusion we studied the anatomical and physiological extent of the development of an accessory circulation to the myocardium involved. The conclusion was reached that a large new collateral circulation developed in many dogs and was sufficient to maintain essentially normal myocardial activity in the zone previously rendered ischemic.

The criteria for such a belief were the experimental findings that 1, röntgenograms as well as macroscopic and microscopic examination showed enlargement of pre-existing or new anatomical channels between the occluded and non-occluded coronary arteries, together with a minimum of infarction; 2, the peripheral coronary pressure curve rose chiefly during systolic ejection rather than during isometric contraction as is normally the case; 3, both its diastolic and systolic values were far in excess of normal and approached the prevailing aortic pressures; 4, the peripheral coronary back-flow (recorded by a new type of optical phasic flow meter) took place during both systole and diastole, varied from 8 to 80 cc./min. and changed directly with the aortic pressure and systolic discharge, and 5, visually the myocardium displayed systolic shortening.

The source of this new blood supply has not been quantitatively differentiated between Thebesian, intra- and extracoronary routes, but upon the basis of the experiments to date its origin appears to be largely the other coronaries since injection studies showed large intracoronary communications and the peripheral flow and pressure decreased toward normal values when the other coronaries were temporarily clamped.

*The effects of anesthesia on dogs. Cyclopropane.*[1] ESTHER M. GREISHEIMER, ROBERTA HAFKESBRING and HULDA MAGALHAES (by invitation). Woman's Medical College of Pennsylvania, Philadelphia.

After establishing controls, the effects of repeated one-hour periods of cyclopropane anesthesia have been studied. The results of a series of observations on one dog will be reported at this time. Blood pressure, heart rate, electrocardiogram, hemoglobin, erythrocyte, leukocyte and differential counts, blood sugar, urea clearance, and the excretion of uric acid and plasma bilirubin (as a measure of liver function) have been determined.

Blood pressure and heart rate increase during the administration of the anesthetic, and return to the normal level rather promptly. No abnormalities were evident in the electrocardiograms.

No significant change was noted in hemoglobin and differential counts; the erythrocyte and leukocyte counts increase during the administration of the anesthetic, then decrease, and then show a secondary rise about one week after each administration of the anesthetic.

Blood sugar has increased in every case during cyclopropane anesthesia. It falls promptly, but the general trend of the blood sugars in the controls between periods of anesthesia is upward.

On the days following the various administrations of cyclopropane the urea clearances were about thirty per cent above the controls. Within five to seven days, the clearances had fallen to about seven per cent above the control level, and reached the normal level in three to four weeks.

The uric acid in the urine varies irregularly with repeated administrations of cyclopropane. This suggests beginning liver damage. The tests for plasma bilirubin remained negative.

*Phytotoxic reactions of fresh, frozen and dried blood specimens.* MARY LOU GRUMBEIN (by invitation) and DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md.

The phytotoxic reactions of freshly drawn blood, kept fluid with highly purified heparin (H. W. & D.), and frozen specimens, after thawing, were compared at various intervals. The method employed was the study of the effect of one per cent solutions of the respective samples on root growth of *Lupinus albus* seedlings reared under standard conditions. Fluid blood kept at 60°F. and that kept at 36°F. showed no difference in toxicity except that the former decomposed more quickly. Blood which had been frozen solid and thawed out was definitely more toxic for seedlings than controls of the same concentration of fluid blood, kept just above the freezing-point. Curiously enough, muscle juices and emulsions of brain tissue, similarly frozen and thawed out and tested for many weeks, were found to become progressively less toxic both when studied on plants and when injected in mice. Blood specimens obtained from such abnormal conditions as menstruation, pernicious anemia and pemphigus and frozen retained their toxicity for many weeks and even months. Dried specimens of whole blood or blood serum (prepared by spreading 0.5 cc. of either on a glass slide and allowing it to dry in the dark at room tempera-

[1] This work was aided by a grant from the Penrose Fund of the American Philosophical Society.

ture) retained their characteristic phytotoxic index for three months when kept in the ice-box. Fluid blood saturated with pure carbon monoxide or with illuminating gas and kept frozen in the dark remained toxic for nearly a year. Even dried monoxide blood, kept in the dark at temperatures of 50°F. or lower, exhibited a phytotoxic effect after long periods of time. These findings may have some diagnostic and medicolegal value.

*Properties of C fibers in mammalian visceral nerve.* H. GRUNDFEST and H. S. GASSER. Laboratories of The Rockefeller Institute for Medical Research, New York City.

Threshold spikes in mammalian postganglionic autonomic fibers, which conduct with velocities below 2 m.p.s., last 2 msec. The absolutely refractory period of these fibers in the splenic, cardiac, coeliac, and hypogastric nerves also lasts 2 msec. After a single conditioning action, recovery of excitability is complete at 12 msec., at which time supernormality begins to develop in hypogastric nerves tested *in situ* as well as in the various excised nerves. The subsequent course of recovery follows the sequence of the after-potentials. The larger proportion of nerves developed negative after-potential accompanied by supernormality for 50–70 msec. This was followed by a positivity lasting between 1 and 2 seconds and having associated with it a subnormal phase of excitability. In other nerves the negative after-potential and supernormality persisted for 300–600 msec.

During repetitive activity the maximal spikes increase as much as 30 per cent in height. Positivity begins at first to deepen, but its growth is either checked or outweighed by progressive increases in the amount and duration of the negative after-potential developed in each successive response. The sequence of potentials at the end of a tetanus depends upon the degree of activity. As the frequency and duration of the activity are increased, the positive after-potential grows deeper and longer and at rates of stimulation above 20/sec. differentiates clearly into two portions—an initial sharp notch lasting 1–2 seconds and a later period of lower but longer sustained positivity.

The after-potential cycle of these fibers is, therefore, composed of a negative after-potential developing simultaneously with a first positive after-potential and causing in its wake the production of a late and long-lasting positivity.

The elements involved in the after-potential cycle of mammalian C fibers accordingly are similar to those causing the after-potentials of A fibers, as well as of bullfrog C fibers. The variations in the pattern of developing after-potentials which are characteristic of each fiber group seem due to quantitative modifications of their component elements.

*Comparative effects of a high glucose and a high fructose diet on spontaneous activity, body weight and various constituents of the liver and body of the albino rat.* JOHN HALDI, GEORGE BACHMANN, CHARLES ENSOR (by invitation) and WINFREY WYNN (by invitation). Laboratory of Physiology, Emory University School of Medicine, Atlanta, Ga.

Litter mates of the albino rat at the time of weaning were placed on equicaloric diets, identical in every respect except that one contained 68 per cent glucose, the other the same amount of fructose. Ten days after weaning the animals were put in revolving cages for the remainder of the experi-

ment. The entire experiment extended over 10 weeks. The food intake of litter mates was kept as nearly equal as possible from day to day, and was completely balanced once a week.

The average activity of the glucose-fed animals, expressed as miles per day, was greater each week than that of the fructose-fed animals. The glucose and fructose-fed males ran a total of 136 and 81 miles, respectively, in eight weeks, or an average of 2.43 and 1.96 miles per day; the females 162 and 118 miles, respectively, or a daily average of 2.87 and 2.09 miles.

The average increase in body weight of 10 males on the glucose and fructose diets was 505 per cent and 513 per cent, respectively; of 6 females, 386 per cent and 378 per cent, respectively.

There was no significant difference in the percentage fat, nitrogen, and glycogen content of the body of the animals on the two diets. Since in the experiments with no activity reported elsewhere, there was a larger amount of body fat in the glucose than in the fructose-fed rats, it would appear as if the greater activity of the glucose-fed animals in the present experiments was at the expense of the body fat.

The average weight of the liver of the glucose and fructose-fed animals was 6.074 and 6.735 grams, respectively, or 2.95 and 3.39 per cent of the body weight. Relative to body weight the liver of the animals on the fructose diet was heavier in 15 out of 16 experiments. The percentage nitrogen and glycogen content of the liver was the same with the two diets. The percentage fat content on the glucose diet was 4.73, as compared with 4.08 on the fructose diet, the difference being statistically significant.

*Electrocardiographic changes associated with experimental imbalance of the autonomic nervous system.* G. E. HALL (introduced by V. E. Henderson). Department of Medical Research, University of Toronto, Canada.

A single subcutaneous injection of acetylcholine (Ach.) produces in experimental animals a rapid increase in heart rate. The degree, as well as the maintenance of this tachycardia is different in different species and may be dependent upon the esterase activity of the serum (Hall and Lucas, 1937).

Continuous intravenous injections of Ach. (1:10,000) produce a cardiac acceleration (2–3 times) which is maintained throughout the injection period. No changes in the electrocardiographic complexes are noted. When, however, the continuous intravenous injections of Ach. are continued daily for long periods of time progressive changes in the E. C. G. occur. Conduction time (P–R interval) is increased, bouts of extrasystoles, 2:1, 3:1 and complete heart block frequently occur; auricular fibrillation, with or without block, is a common finding. Changing T-waves occur as the coronary arteries become involved.

When a similar but relative parasympathetic preponderance is produced following adrenalectomy, the E.C.G. shows gradual changes until marked evidence of myocardial ischemia is produced. Absence of P-waves and heart block are early findings. The failure of the heart to accelerate following atropinization is accompanied by a great increase in the voltage of both R and T waves with a continuance of the block. Heart sounds are irregularly spaced, many appearing independent of the ventricular complexes (Hall and Cleghorn). The increase in serum K does not account for these E.C.G. changes.

Double stellage ganglionectomy renders the heart incapable of respond-

ing chronotropically to graded vagal stimulations (thyratron)—an effective stimulus resulting in complete inhibition.

*Effect of administration of adrenal cortical hormone preparations on litter size and weight in the normal rat.* V. E. HALL, P. E. CHAMBERLIN (by invitation) and O. H. MÜLLER (by invitation). Department of Physiology, Stanford University, Calif.

Normal albino rats given 0.8 rat unit of adrenal cortical hormone daily throughout pregnancy on a diet containing 0.37 per cent sodium and 0.37 per cent potassium produce litters of the same size and weight as controls on the same diet. However, on a diet containing 0.07 per cent sodium and 0.68 per cent potassium, which in controls significantly reduces litter weight, adrenal cortical hormone administration restores the value observed in animals on the diet of more normal mineral composition.

*The effects of intravenous injections of acacia upon certain functions of the liver.* W. KNOWLTON HALL (introduced by R. B. Gibson). Department of Biochemistry, Louisiana State University Medical Center, New Orleans and the Pathological Chemistry Laboratory, State University of Iowa Hospital, Iowa City.

Repeated intravenous injections of gum acacia solution in dogs have resulted in evident damage for carbohydrate and serum protein metabolism functions of the liver. These changes were shown by glucose and galactose blood sugar tolerance curves and determinations of the plasma proteins. Alteration of the clotting mechanism of the blood, especially the lowered fibrinogen content, has resulted in failure to clot, or in formation of small, fragile and fibrous clots and in a longer bleeding time. The removal rate of bilirubin injected into the blood was unaffected.

*Precocious masculine behavior following administration of synthetic male hormone substance.* JAMES B. HAMILTON (introduced by Harold E. Himwich). Yale University School of Medicine. (Motion picture.)

Moving pictures will be used to illustrate the cock-like behavior induced after 8 daily injections of testosterone propionate[1] to newly-hatched chicks. At a body weight of less than 70 gm. chicks execute complicated coördinated actions such as crowning and strutting. Crowing involves the characteristic movements of head, neck, body and tail accompanied at the proper time by vocalization in the form of a shrill canary-like monotone. Presumably the as yet underdeveloped syrinx does not lend itself to the tones and variations of the adult call.

This cock-like behavior is interpreted as evidence that a behavior recognized as "masculine" in the chick can be stimulated by synthetic male hormone substance, and that these phenomena are irrespective of any knowledge of the individual that treatment is being given. Possible significance of these data is discussed with regard to behavior changes appearing in human castrates who have received administration of this androgenic material.

*Blood pressure values in street dogs.* W. F. HAMILTON and E. R. PUND (by invitation), A. W. SIMPSON, JR. (by invitation), G. M. COLSON (by

[1] Furnished under the tradename Perandren by the Ciba Company.

invitation) and H. W. COLEMAN (by invitation). Department of Physiology and Pharmacology and the Department of Pathology, University of Georgia School of Medicine, Augusta.

Using the hypodermic manometer the blood pressures of 130 street dogs have been determined. The animals were lightly morphinized and pressures recorded from the femoral arteries. The systolic pressure ranged from 100 to 275 mm. Hg, the diastolic from 30 to 140, the dicrotic from 85 to 185. The means of these three measurements were 182, 94, and 132 mm. Hg.

Males and females showed almost identical averages.

From the appearance of the teeth the dogs were divided roughly into 5 age groups. The group of dogs with immature teeth had pressures of 160/64. The other groups presented values not significantly different from the mean of the group as a whole.

Femoral systolic pressures are much higher than carotid systolic pressures. Femoral dicrotic pressures are not much lower. These facts and the wide range in different dogs will explain the contradictory values reported from different laboratories.

Two dogs were trained to submit to arterial puncture without morphine. Their pressures under morphine before training were about the same as after training without morphine. The heart rate under morphine was much slower than without. One dog changed his basal level from 150/80 to 180/105 during a year's observation. Morphine produced a slight lowering of pressures in trained dogs.

Pathological examination of the tissues of 17 unselected dogs was made. Dogs were said to have hypertensive lesions when arteriolar sclerosis was observed in the kidney and usually in the liver and pancreas, associated with hypertrophy of the small arteries in the same organs. Glomerular nephritis would also indicate hypertension.

Of the 17 dogs 8 had hypertensive lesions, 5 with high pressures and 3 with low pressures. Among those with low pressures one had questionable lesions and two were very old dogs with hypertrophied hearts and extreme lesions. Among the 9 dogs without hypertensive lesions, only two had high blood pressures.

Average blood pressures increased in groups having progressively larger heart weight—body weight ratios. The group having the largest hearts, however, showed senile changes and low pressures.

Aid in pursuance of this investigation is acknowledged from N. Y. A. and from the American Medical Association.

*Stimulation of the cold end organs of the human skin by radiation.* JAMES D. HARDY and T. W. OPPEL (introduced by E. F. Du Bois). Russell Sage Institute of Pathology in affiliation with the New York Hospital and Department of Medicine, Cornell University Medical College, New York City.

Using the radiation technique which we have described, the end organs in the skin which are sensitive to cold were stimulated. The source of "cold radiation" was a block of solid $CO_2$, 25 cm. times 25 cm. The rays were concentrated onto the skin by a large, silver cone, and a five fold increase in intensity obtained. The foreheads of ten subjects were tested and the individual sensitivity to "cold radiation" found to be approx-

imately the same. Two subjects were studied in detail by determining the minimum stimulating intensity of "cold radiation" for areas of different sizes. Areas smaller than 3 $cm^2$ could not be stimulated. The size of the exposed area was increased so as to include the entire upper part of the body.

The thermal changes produced on the skin surface by these radiations were measured to 0.01°C. with a radiometer.

The results of the experiments are:

1. The number of cold end organs per unit area is greater than the number of heat endings.
2. Spatial summation of cold sensation follows the same laws as heat sense, but the summation is poorer.
3. The threshold of thermal stimulation for a cold end organ is a fall in skin temperature of 0.004°C. per second. This skin temperature change is more than five times as great as that necessary to stimulate a heat ending.
4. Cold radiation produces about twice the change in skin temperature, calorie for calorie, as does heat radiation.
5. Temperature sensation does not depend upon vascular changes in the skin.
6. Heat and cold sensation are not mediated by the same end organ.

*The receptive fields of optic nerve fibers.* H. K. HARTLINE. Johnson Foundation, University of Pennsylvania, Philadelphia.

The region of the retina which must be illuminated to produce a response in a particular optic nerve fiber is termed the receptive field of that fiber. Its boundaries may be mapped by a very small spot of light, the position of which can be accurately controlled. The location on the retina of the receptive field of a given fiber is fixed; its extent depends on the intensity and area of the exploring spot, and on the state of adaptation of the eye The receptive field of any fiber is smallest for threshold stimulation, being then usually 1/4 to 1/2 mm. in diameter. With intensities 100 to 1000 times threshold it may be twice this size, although the strongest responses are always obtained from the more sensitive central region of the field.

Fibers responding at both "on" and "off", or at "off" only, also respond to movement of a spot of light, provided it falls within the receptive field of the fiber under observation. Likewise the shadow of a thin wire produces responses whenever it is moved within the receptive field; this proves that these spatial effects are not due solely to scattered light, but that there is true physiological convergence upon the ganglion cell. The larger the area of a spot falling within the receptive field, the greater is the response in the fiber; two spots illuminated together produce greater effects than either one alone. There is, therefore, summation within the receptive field. The "off" response from a spot can be inhibited by illumination of an adjacent spot within the receptive field. There is, therefore, convergence of inhibitory as well as of excitatory effects in the retina.

*The refractory state produced by repeated injections of adrenal extract.*[1] F. A. HARTMAN, LENA A. LEWIS (by invitation), and KENNETH P. MCCONNELL (by invitation). Department of Physiology, The Ohio State University, Columbus.

[1] Aided by a grant from The Rockefeller Foundation.

We have previously shown that repeated intravenous injections of adrenal extract develop a refractory state in normal subjects (F. A. Hartman, L. A. Lewis, C. G. Toby, Science **86:** 128, 1937). This state is one in which the characteristic electrolyte response fails to develop upon injection of extract.

Unlike intravenous injections, repeated subcutaneous injections fail to produce a refractory state. As many as 9 subcutaneous injections of 80–160 C.U. each, into normal male dogs over a period of 4 to 6 weeks failed to produce any indication of a refractory state.

Normal female dogs which were refractory to beef adrenal extract, i.e., gave no electrolyte response to 40–50 C.U., responded with typical electrolyte changes when injected with pig adrenal extract in similar doses.

*Urine electrolyte excretion, dog X*

| | VOLUME | pH | M.EQ. PER 6 HOURS | | | |
|---|---|---|---|---|---|---|
| | | | Na | K | $NH_3$ | T.B. |
| | cc. | | | | | |
| Controls (average 4 experiments) | 128 | | 11.17 | 6.60 | 6.50 | |
| 1st injection beef adrenal extract, 20 C.U. | 80 | | 2.15 | 6.70 | 10.10 | |
| Repeated injections intravenously over a period of weeks of 20–160 C.U. beef adrenal extract | | | | | | |
| Controls (average 5 experiments) | 134 | 5.9 | 13.19 | 4.16 | 9.30 | 29.43 |
| Final injection, beef adrenal extract, 40 C.U. | 160 | 6.0 | 11.88 | 4.96 | 8.70 | 32.90 |
| Injection, 48 C.U. pig adrenal extract | 96 | 6.0 | 3.93 | 5.60 | 9.90 | 27.43 |

*Endocrine and somatic factors concerned in the time of vaginal canalization in the rat.* HANS O. HATERIUS. Department of Physiology, The Ohio State University, Columbus.

Rats from 22 litters were subjected to bilateral oophorectomy at ages ranging from 8 to 31 days; controls were comprised of operated and unoperated littermates. In a second series of 8 litters, in addition to castrates, autotransplantation of ovaries was performed in one or more animals per litter, again leaving two or more unoperated and/or operated controls. Following canalization vaginal smears were made to check graft viability. 161 animals in all were utilized, including 30 litters. The vaginal orifice was established in spayed animals at an age ranging from the 54th to the 114th day, the average falling at 75.73, as compared with a range in controls extending from the 39th to the 64th day (average 47.41). Vaginal opening occurred in autotransplanted animals within the range of controls. In all cases the grafts recovered displayed characteristic follicular development, which bears out the concept that the endocrine function of the ovary is an important factor in the earlier date of vaginal opening in the intact animal. Age at time of ovarian ablation is of prime importance, moreover, since the greatest disparity in ages between castrates and controls appeared in animals which had been oophorectomized early in life—at ages ranging from 8 to 27 days; beyond the age of 31 days the difference decreased sharply. This was verified in an additional series of 11 litters, spayed at ages of 35 to 45 days; these animals failed to reveal any significant lag, a result not unexpected since animals in this age-range

are approaching sexual maturity. It emphasized, however, the fact that ovarian factors are active for some time before the outspoken changes attendant upon puberty manifest themselves.

Weights and sizes of castrates and controls differed as strikingly as did the dates of opening. Controls never attained the weights, for example, shown by castrates at time of opening, although the latter showed considerable weight variation. Body lengths, however, at the time of opening showed close agreement with those reported recently by Engle *et alii*.

It seems likely, therefore, that the time of vaginal canalization in the intact animal is regulated both by ovarian and growth factors, the endocrine being superimposed upon the somatic. When the endocrine factor is eliminated vaginal opening is delayed, since it is then dependent upon the somatic factor alone. The earlier the ovarian endocrine influence is removed, naturally, the more dependent is the process upon body growth alone.

*The effect of parathyroid hormone on the diffusibility of magnesium into the peritoneum.* VICTOR G. HAURY (by invitation), A. CANTAROW and C. G. WHITBECK (by invitation). Departments of Pharmacology and Medicine, Jefferson Medical College, and the Laboratory of Biochemistry, Jefferson Hospital, Philadelphia.

Twelve dogs were given intraperitoneal injections (100 cc. per kgm.) of 2.5 per cent glucose and 0.9 per cent saline. Samples (20 cc.) of blood and peritoneal fluid were withdrawn at hourly intervals for the first 5–8 hours and again at the end of 16 hours and analyzed for Mg, Ca and P. In 8 of these dogs, 500–600 units of parathyroid hormone (obtained through the courtesy of Dr. E. A. Sharp of Parke, Davis and Co.) were injected intramuscularly at the end of the 16 hour period and samples of blood and peritoneal fluid again withdrawn hourly for 5–8 hours and analyzed.

The fluid-serum Mg ratio at the end of the 16 hour period ranged from 57 to 87 per cent (average 75). In the 8 dogs receiving parathyroid hormone the fluid-serum Mg ratio rose to 68–110 per cent (average 88). In 5 experiments parathyroid administration was followed by a progressive fall in serum Mg (0.35–0.81 mgm. per 100 cc.) with a tendency to return to normal in 4–6 hours. In 2 instances the serum magnesium rose progressively (0.4 and 0.7 mgm. respectively). In one animal no significant change was noted. The Mg content of the peritoneal fluid increased steadily following the injection of the parathyroid hormone, reaching equilibrium in about 5 hours.

In 2 animals previously subjected to repeated filling and drainage of the peritoneal cavity (with glucose and saline solution), parathyroid injection was followed by a small initial drop in serum Mg (0.3 mgm.) which returned to normal in 4 and 6 hours respectively. The fluid-serum Mg ratio in both these animals increased during the first 2–4 hours and then decreased.

Since the fluid-serum Mg ratio increases following parathyroid administration in spite of the drop in serum Mg, it is possible that parathyroid in some way increases the diffusible fraction of serum Mg.

*Observations on the secretion of dye by the isolated fish liver.* CHARLOTTE HAYWOOD and VIRGINIA C. DICKERSON (introduced by R. Höber).

Physiological Laboratory, Mount Holyoke College, South Hadley, Mass., the Bergens Museum Biologiske Stasjon, Herdla, Norway, and the Marine Biological Laboratory, Woods Hole, Mass.

The demonstration by Höber and his co-workers that the isolated frog liver is able to concentrate dyes furnished to it in perfusion has been extended to the isolated fish liver. Perfusion with oxygenated modified Ringer's solution was maintained via the portal vein at a pressure of about 8–10 cm. water; collection of secretion was made by cannulating the gall-bladder at its beginning.

The concentration factor, $\frac{\text{per cent dye in secretion}}{\text{per cent dye in perfusion fluid}}$, was determined with the administration of 0.0005 per cent eriocyanin, a sulphonic acid, lipoid-insoluble dye. Maximum values obtained with salt-water fishes were:—Tautoga onitis, 558; Labrus berggylta, 397; Labrus mixtus, 612; Gadus pollachius, 336; Lophius piscatorius, 664. As examples of fresh-water fishes, two species of trout gave the following maximum values:—Salmo fario, 3337; Salmo irideus, 3046.

Further studies upon the trout liver showed that the concentration factor is lowered by increasing the amount of dye administered. When 0.005 per cent and 0.05 per cent eriocyanin were used, the average concentration factors were found to be roughly one-tenth and one-hundredth, respectively, of that obtained with 0.0005 per cent eriocyanin. A similar trend, although far less marked, was seen in the average maximum rate of fluid secreted during perfusion: with 0.0005 per cent dye, 24.9 cu. mm. per hour; with 0.005 per cent dye, 8.5 cu. mm. per hour; with 0.05 per cent dye, 1.9 cu. mm. per hour.

Other dyes, of varying physical and chemical properties, gave the following maximum concentration factors, when administered to the trout liver in 0.0005 per cent concentration: a sulphonphthalein dye, bromphenol blue, 1963; two sulphonic acid dyes, Congo red, 706, and erioglaucin, 927; three carboxylic acid dyes, methyl red, 600, chromviolett, 1009, and phloxin J, 1880; a basic dye, neutral red, 1077. The secretion of Congo red and of chromviolett is of further interest because of their colloidal character.

It is concluded that certain fish, especially trout, are suitable material for studies upon the secretion of dyes by the isolated liver.

*The assay of the oestrus-inducing gonadotropic principle in the dietary anoestrus adult rat.* R. D. H. Heard and S. S. Weinstein (introduced by C. B. Weld). Connaught Laboratories and Department of Biochemistry, University of Toronto, Canada.

The chief disadvantages of the familiar infantile rat test for the oestrus-inducing gonadotropic principle are variation in sensitivity with age and degree of accuracy attained. In most laboratories the latter is seriously restricted by the limited number of available young females of the same age. A system of assay using the dietary anoestrus adult rat (vitamin B deficiency) has been evolved which largely overcomes these objections and provides several additional advantages. The results of more than 1500 individual determinations establish the suitability of the test object.

The well known procedure of considering the percentage response of an adequate number (20) of animals at varying dose levels has been followed. Subdivision of the dose is unnecessary; vaginal cornification persists for

24 hours or more following a single injection of active extract. Age, degree of avitaminosis and body weight (within comparatively wide limits) are without influence. A number of successive tests (at least nine) can be carried out on the same group without appreciable disturbance in sensitivity. Dosage-response curves have been constructed for three strains of rats and have been analyzed by statistical methods.

A comparison of the infantile (21 days of age) and mature rat tests in animals of the same strain shows that the individual susceptibility of the adult animal and the general sensitiveness of that method are considerably greater. The respective slopes (with their standard errors) of the transformed dosage-response curves for the infantile rat using (a) the technique of a single injection (210 observations) and (b) the divided dose (3 portions; 262 observations) and (c) for the mature animal (single injection; 561 observations) have been determined as $3.14 \pm 0.92$, $5.45 \pm 0.68$ and $8.72 \pm 1.03$ whence the limits of error (for 20 animals at $P = 0.99$ expressed as percentages of the true result) are 28 and 352 per cent, 70 and 143 per cent, and 76 and 132 per cent for the three methods. The logarithms (with their standard errors) of the amount of a standard preparation required to induce oestrus in 50 per cent of the animals injected are $1.364 \pm 0.047$, $0.966 \pm 0.016$ and $0.525 \pm 0.008$ whence the values of the rat unit are 23.1, 9.24 and 3.35 gamma respectively.

*The visual functions of a completely colorblind person.* SELIG HECHT, SIMON SHLAER (by invitation), EMIL L. SMITH (by invitation), CHARLES HAIG (by invitation) and JAMES C. PESKIN (by invitation). Laboratory of Biophysics, Columbia University, New York City.

In previous researches we have studied such visual functions as intensity discrimination, flicker, visual acuity, and dark adaptation for normal eyes. To complement these studies we have now investigated the same functions with the eye of a completely color-blind person.

The sensibility distribution in the spectrum has a maximum near 520 $m\mu$ at all brightnesses, and thus probably corresponds to rod vision alone. This is confirmed by studies of dark adaptation which yield final thresholds like those usually found for rod vision. Dark adaptation, measured both centrally and peripherally in the retina, is a single continuous function, and regardless of how bright the preceding light adaptation has been, is of the rapid type only, such as is found for normal eyes following low light adaptation.

Both intensity discrimination and critical fusion frequency when measured over the whole light intensity range show two sections, one at low intensities and the other at high intensities with a fairly sharp transition from one to the other. Intensity discrimination as given by the value of $\Delta I/I$ is as good as for the normal eye, and covers much the same range. Flicker function of the low intensity range is also like normal. The high intensity flicker function, however, is strongly depressed so that the maximum critical frequency is only about 20 cycles per second compared to 50 cycles for the normal.

Of the two sections shown by the colorblind data for flicker and intensity discrimination, the low intensity one clearly corresponds to normal rod function. The high intensity portion, however, does not correspond to cone function even of a rudimentary kind, because its position on the intensity scale relative to the rod portion cannot be changed by using light

from different parts of the spectrum. This shows that the spectral sensibilities of the elements concerned at high intensities is the same as those functional at low intensities, and agrees with the independent measurements of spectral sensibility.

It is possible to explain these measurements by supposing that our colorblind possesses two kinds of rods having the same photosensitive substance in them but distinguished by having different thresholds due perhaps to a difference in the concentration of sensitive substance. There are indications that even normal persons may possess these two types of rods.

*Inhibition of parturition in the rabbit by the injection of estrogenic hormone.* GEORGE P. HECKEL (by invitation) and WILLARD M. ALLEN. The University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The corpora lutea are necessary for the maintainance of pregnancy in the rabbit, and there is considerable evidence that their decline is necessary for parturition to occur. Since it has been shown (Science, **84**: 161, 1936) that injection of an estrogen in pseudopregnancy in the rabbit causes the corpora to persist in a functional state beyond their usual time, it might be expected that injection of an estrogen in the latter part of pregnancy would inhibit parturition by similarly maintaining the corpora lutea. This has been found to be the case.

In the experiments rabbits were given repeated injections of estradiol monobenzoate (Progynon B) in the latter part of pregnancy. Daily doses of 110–195 i.u. per kilo, beginning as late as the 31st day, caused retention of the fetuses beyond the 32nd day, the usual time for delivery, in nearly every case. In some animals dead fetuses were retained as late as the 52nd day, when the animals were killed. In others delivery of all or part of the litter occurred some time after term in spite of continued injections. When the ovaries were examined histologically following delayed parturition, the corpora lutea were found to appear much like those of normal pregnancy. Normally the corpora degenerate shortly after delivery.

Post-mature fetuses were not obtained; in the experiments so far carried out, doses of estrogen sufficient to delay parturition also caused death of the fetuses.

In order to determine whether the presence of the ovaries is necessary for the inhibition of parturition, several pregnant animals were castrated on the 27th day when injections of estrogenic hormone were begun, and others were castrated after injections had been given for a few days. In all cases delivery occurred within three days after castration despite continued injections.

It is concluded that the inhibition of parturition effected by the injection of estrogenic hormone is due to maintainance of the corpora lutea.

*The hydrostatic effect of gravity on the circulation in supported, unsupported and suspended postures.* FRANCES A. HELLEBRANDT and ELIZABETH BROGDON (by invitation). University of Wisconsin, Madison.

The slight but continual shifting of the center of mass of the body in quiet standing may produce a squeezing action of anti-gravity muscles of possible aid to the venous return in the vertical posture. The development in this laboratory of techniques for measuring postural sway suggested

the evaluation of this accessory pump in the circulation. We have first been concerned with the response of the circulation to conditions of different hydrostatic handicap. Seven young adult women acted as subjects. Their cardiovascular response to 15 minutes of normal standing was noted in a series of 35 experiments. In no case did collapse occur, though the blood pressure readings fluctuated widely with occasional extreme narrowing of the pulse pressure. The heart rates were well above the recumbent level and oscillated considerably. Cardiovascular observations were made in 14 experiments in which the subjects were suspended motionless in water at 36°C. for 20 to 60 minutes. The readings were remarkably steady. The systolic and diastolic pressures were generally slightly lower than in standing and approximated the recumbent level. The heart rate was consistently lower than the standing rate, but higher than the recumbent. With a tilting board each subject was passively moved to the vertical, being supported for 5 minutes at 15, 30, 45, 60, 75 and 90 degrees with the horizontal. When allowed to rest horizontally between each position, the cardiovascular reactions were adequate and no collapse occurred. Three subjects were held for 15 minutes at each angle progressively without rest periods and were brought to the point of collapse at 45 or 60 degrees. The 75° angle was tolerated more easily and showed less marked effects on the circulation, for even though supported against the board, the subject here carried enough weight on her lower extremities to aid the venous return through muscle action.

*The graphic registration of shifts in the center of weight during standing.* FRANCES A. HELLEBRANDT and L. E. A. KELSO (by invitation). University of Wisconsin, Madison. (Demonstration.)

The method originally described in Science (**86:** 451, Nov. 12, 1937) has been modified to increase the usefulness of the compound watt oscillograph for the analysis of postural sway. By standing upon the upper of two beams placed at right angles to each other and resting each upon platform scales equipped with levers for the indication of changes in spring tension, shifts in the center of weight occurring in the cardinal vertical orientation planes may be recorded as a moving kymogram. A secondary is suspended from each lever so as to hang freely in the air-gap of a slotted core transformer in which the flux density is practically uniform. The current induced in the secondaries is led to two slow speed watts units so mounted that any rotation of one vibrator is projected to and compounded by the other.

The axes of rotation of the vibrators are perpendicular to each other. The shuttle for vibrator 1 is a part of the frame supporting the shuttle of vibrator 2. The frame for vibrator 1 is itself supported on frictionless bearings. It is free to rotate about its long axis which coincides with the axis of rotation of the shuttle. The coil of vibrator 2 is in the form of a circle so fastened in the shuttle that its axis of rotation is a diameter. Thus the instrument may be used to compound any two rectilinear motions, directly or proportionately, as if they were perpendicular to each other. The compounded motion of shuttle 2 is recorded on a stationary sheet of kymograph paper by a long slender pointer which hangs vertically from the shuttle in zero position. Frictionless contact between pointer and paper is made by a light needle mounted to allow free movement in an axial direction. Since the average torque due to field flux and vibrator current

is proportional to the secondary movement and since this in turn is proportional to changes in scale load, the kymographic reproduction of the vibrations of the oscillograph needle reflect every shift in the center of weight during standing.

*Heat tolerance of dogs.* ALLAN HEMINGWAY. Department of Physiology, University of Minnesota Medical School, Minneapolis.

Trained unanesthetized dogs have been heated by measured dosages of diathermy heat for a three hour period. The rate of heating has been made equal to the basal metabolic rate or an integral multiple of this. The tolerance limit has been measured by the development of respiratory alkalosis as indicated by the plasma pH and $CO_2$ content. Respiration changes and gaseous metabolism determinations have been made. Respiratory alkalosis occurs with a heat dosage of two to three times the b. m. r. at an environmental temperature of 30°C.

*Respiratory variations in the weight of a man submerged in water, and their significance.* J. M. TURNER (introduced by Yandell Henderson). Laboratory of Applied Physiology, Yale University, New Haven, Conn.

A live man of youthful build with his lungs inflated to resting inspiration almost floats in fresh water. After death he sinks even in salt water (sp. gr. 1,025).

For a man of 60 kilos these facts indicate a decrease of 1.5 liters or more in the volume of the thorax. The reason for this deflation lies in the fact that at death the thoracic muscles and diaphragm lose the tonus which throughout life keeps the lungs expanded. It is known that in states of depression after surgical operations and in illness the vital capacity is decreased; but it is probable that from birth to death, even in the deepest expiration, the diaphragm is never completely relaxed. In agreement with W. R. Hess (Die Regulierung der Atmung, Leipzig, 1931) we regard the movements of respiration as essentially variations of tonus level.

Weighing the body in water affords a convenient method of studying variations of lung volume. It has the advantage over a body plethysmograph that the air around the body in a plethysmograph becomes warmer, expands and therefore introduces errors that are not easily controlled.

In fresh water a variation of weight of one kilo corresponds exactly to a converse variation of one liter of volume.

We are using this method to determine the inspiratory and expiratory volumes after inhalation of carbon dioxide.

*Studies in differential blood pressure.* J. F. HERRICK, JOSEPH B. CADY (by invitation), EDWARD J. BALDES and FRANK C. MANN. Mayo Clinic and Mayo Foundation, Rochester, Minn.

The purpose of this investigation was to study the relationship that exists between the differential blood pressures (the difference in systolic pressures in the various arteries of the body) and the effective peripheral resistances. Changes in the latter resistances were brought about by histamin, acetylcholin, ephedrin, epinephrin, certain anesthetics and regional sympathectomy.

The Hamilton "hypodermic" manometer was used for measuring blood pressures. In a few instances these measurements were compared with values obtained by the ausculatory method.

An index of the effective peripheral resistance in any given blood vessel is obtained by the ratio of the mean blood pressure to the average blood flow in that vessel. The blood flows in the femoral and carotid arteries or, in the femoral and brachial arteries, were measured (Thermostromuhr method) simultaneously while the blood pressure was observed. From these data the relative effective peripheral resistances are determined.

It is found that the effective peripheral resistance in the femoral artery is considerably greater than that in the carotid artery when the dog is lying quietly on the table. Under these conditions the differential pressure in these arteries is about 30 mm. Hg. The intravenous injection of a small amount of histamin causes the effective resistances in these two arteries to become almost equal and, at the same time, the differential pressure approaches zero.

The conclusions of several experiments indicate that the differential pressures are, (a) either abolished or reversed by acetylcholin and by histamin, (b) markedly augmented by ephedrin and epinephrin, (c) significantly diminished by certain anesthetics and by lumbar sympathectomy.

The rôle of effective peripheral resistance in differential blood pressure will be discussed.

*The influence of vitamin A upon renal clearance in dog and man.* R. C. HERRIN, H. J. NICHOLES (by invitation) and K. L. SIEBECKER (by invitation). University of Wisconsin, Madison.

Dietary vitamin A deficiency as developed in 9 young dogs within 6 to 10 weeks resulted in urea clearances of 18 to 24 cc./min./M$^2$. They spontaneously recovered with clearances of 40 to 45 cc./min./M$^2$. Two to 6 months later, the clearances ranged from 21 to 30. Thirty-seven simultaneous urea-inulin clearances on 4 dogs gave an average urea/inulin ratio of 0.54, indicating that the lowered clearance was due to reduced filtration. In avitaminosis A, the dogs are slower in starting and do not maintain diuresis as well as when normal. With large amounts of water they are capable of urine flows of over 10 cc./min./M$^2$. No vitamin A or carotene was found in the plasma. The urine showed many epithelial and red blood cells, albuminuria but no casts. Near terminus the urea clearance rose above 80 with a corresponding rise in inulin clearance. In 6 adult dogs, after 5 months on vitamin A deficient diet, the urea clearance was 24 to 28. In one dog, 12 days post-partum it decreased to 50 per cent of normal.

Vitamin A administration to 12 dogs, after 10 to 60 days, resulted in urea clearances of 45 to 61 cc./min./M$^2$. The average urea/inulin clearance ratio was 0.57. The clearance remained elevated in some dogs for 6 weeks after vitamin A administration ceased. In 3 dogs, apparently an excess of carotene caused impairment of appetite and a reduction of clearance to normal.

The normal diet of 3 human subjects, 2 of whom had normal dim vision by the bio-photometer test and one whose vision was at the low edge of normal, was supplemented with 50,000 to 90,000 units of vitamin A. In 58 days, the average increases in urea clearance were respectively 20, 34 and 60 per cent. The urea/creatinine ratio averaged 0.62. Dim vision returned to normal in one but did not increase in the normal subjects.

Although the kidneys possess 65 per cent of their function in avitamino-

sis A, it appears that vitamin A normally plays an important rôle in renal physiology.

*Some observations on the circulation in several skin areas with a photoelectric plethysmograph.* ALRICK B. HERTZMAN. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

A beam of light falling on the skin penetrates some distance and scatters so that a sufficiently intense transillumination extends far enough beyond the point of incidence of the light to permit adaptation of the photoelectric plethysmograph to the study of the skin circulation of the arm, leg, face, scalp, nose and ear. Application to the skin of the trunk is mechanically complicated by the breathing movements. The photoelectric oscillations with the pulse, vasomotor effects, etc., are recorded on the electrocardiograph after amplification. Semi-quantitation is provided by a filter carrier mounted between the photocell and the skin.

The volume pulse so recorded varies in amplitude in comparable skin areas of different individuals and in different skin areas of the same individual when comparisons are made on the resting subject, reclining in bed, warmly covered with blankets. A heavy smoker, otherwise normal, showed very small volume pulse waves. The wave size is much greater in the skin of the face and ear than in that of the leg and fore-arm. The wave size in the latter is the least of all areas observed. No attempt has been made to correlate wave size with vessel counts in the skin, with skin thickness, or with proximity to skeletal muscle. The wave form generally resembles that of the peripheral arterial pulse which may be inadvertently recorded if the plethysmograph is placed over a main artery such as the radial.

"Spontaneous" changes in blood content such as those observed in the fingers have also been observed prominently in the skin of the forehead, ear, nose, lips, cheek, foot, and less so in the fore-arm and thigh. The skin vessels of the fore-arm dilated during sleep, constricting sharply on awakening. The Valsalva experiment engorged all skin areas observed, obliterating the pulse waves. Amyl nitrite dilated the skin vessels of the thigh and forehead, but "passively" constricted those of the fore-arm. Smoking constricted the latter.

*Photoelectric plethysmography and sphygmography in man and animal.* ALRICK B. HERTZMAN. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo. (Demonstration.)

Photoelectric techniques for the detection of changes in the blood content of tissues and of the pulse are exhibited.

The plethysmographs are designed for use on the fingers, toes, nasal septum and skin of man and on any tissue which may be satisfactorily transilluminated in the animal. They all operate on the principle that the capacity of tissues to light varies with the blood content. Resultant variations in the photoelectric currents are recorded on the electrocardiograph after amplification. The techniques provide opportunity for the study of the details of the volume pulse as well as more slowly developing changes in tissue blood content. Plethysmograms illustrative of various peripheral vascular conditions (physiological and pathological) are also exhibited.

The photoelectric sphygmographs also use the electrocardiograph for

recording. They operate on the principle that if a beam of light falling simultaneously on two photocells (voltaic type) connected in opposition, moves from one cell towards the other, current relations are correspondingly altered and readily recorded on the electrocardiograph. This principle may be used in conjunction with transmission sphygmographs or with mirrors mounted directly over the artery to detect the pulsations. The principle may also be used as a convenient way of magnifying and recording other oscillations as those obtained with membrane manometers of the Hamilton type. Illustrative records are exhibited.

*Some electrocardiographic findings in early hypertension.* L. M. Hickernell and I. L. Ershler (introduced by J. S. Robb). Department of Pharmacology and the Student Health Service, Syracuse University, N. Y. (Read by title.)

During the routine examinations at the Student Health Service of Syracuse University numerous students of college age were found to have a persistently elevated blood pressure with the absence of any other physical abnormality. Complete laboratory and clinical study of these students has been made. The electrocardiograms show certain minor deviations from the normal. These deviations, however, are consistent throughout the group and seem to have more importance than has been given them heretofore. The alterations consist mainly of slight R-T slurring, slight R-T displacement from the iso-electric level and left axis deviation. The regularity of the appearance of these deviations leads us to suggest that the electrocardiograms may be significant of changes developing far earlier than other clinical manifestations of vascular disease. We are indebted to Dr. J. S. Robb for directing this study and for providing valuable technical assistance.

*Metabolism of depancreatized dogs and cats following bilateral ligation of the lumbo-adrenal veins.* H. E. Himwich, J. F. Fazekas (by invitation) and S. J. Martin. Department of Physiology and Pharmacology, Albany Medical College, Albany, N. Y.

The oxidation of carbohydrate by depancreatized animals has been demonstrated after hypophysectomy by Houssay, Chambers and their associates. These workers observed utilization of glucose as well as a respiratory quotient indicating combustion of some carbohydrate in such a preparation. The ability of cats and dogs subjected to pancreatectomy and bilateral ligation of the lumbo-adrenal veins to oxidize carbohydrate was determined in the present study. Animals so prepared are capable of oxidizing a certain amount of carbohydrate, as shown by the following. 1. Though the blood sugar is usually raised (approximately 300 mgm. per cent), yet on fasting these animals become aglycosuric and finally may succumb to hypoglycemia. 2. Fasting D/N ratios, as well as those after the ingestion of meat, are significantly lower than the ratio characteristic of pancreatic diabetes. 3. Four dogs received 25–40 grams of glucose daily. Urinary studies disclosed an average utilization of 7, 11, and 16 grams in 3 of these animals. The remaining one was incapable of utilizing any ingested carbohydrate. 4. The oxidation of carbohydrate is further indicated by a respiratory quotient above 0.7, the range being from 0.75 to 0.87. 5. The ketonuria is slight, and blood fat values are usually within the normal range. It is concluded that the condition of chronic adrenal

insufficiency renders the depancreatized animal capable of oxidizing additional carbohydrate.

*Skin temperature studies on sympathectomized and deafferented cats.* J. C. HINSEY and R. A. PHILLIPS (by invitation). Department of Physiology, Cornell University Medical College, New York City.

The Hardy radiometer has been modified so as to make it available for skin temperature studies on experimental animals. This has been accomplished by the reduction of the area of skin required for accurate measurement to 0.75 sq. cm. At a constant room temperature of 20.5°C., the animals were placed on their backs in a hammock. Care was taken to insure adequate circulation to the limbs at all times.

The animals were studied after a one-stage operation in which the entire right sympathetic chain (from just rostral to stellate ganglion to below S 1 ganglion) and the left abdominal sympathetic chain were excised; and also after the removal of the remaining left thoracic sympathetic chain. Two months after the first operation the pads of the operated fore limbs were about 3°C. warmer than the normally innervated fore limb pads. After the second stage operation there was less than 1°C. difference in the temperature of the pads of the two extremities within the first week. After intravenous injection of 1 cc. of 1/100,000 solution of epinephrine, the temperature decreased equally in the pads of the two sides by about 4°C. No evidence of a difference in sensitization was observed within 8 days after the second stage operation. The typical differences in sensitization of the nictitating membrane and pupil were observed, however.

After complete deafferentation of one upper extremity, there was no evidence that the pads of the operated limb extremity were colder than the normally innervated one. This was true a few days after deafferentation and as long as 13 months following operation.

*The cause of the fall in alveolar $CO_2$ during quiet standing.* FRED A. HITCHCOCK and J. K. W. FERGUSON. Department of Physiology, The Ohio State University, Columbus.

In an extended series of observations it was found that on standing after a period of reclining there was invariably a decrease of 7 to 15 per cent in the alveolar $CO_2$. This confirms the observations of Higgins (1914), Turner (1927) and Main (1937). This fall in alveolar $CO_2$ cannot be attributed to overventilation and consequent washing out of $CO_2$ from the blood since observations on the respiratory exchanges show that: 1, the R. Q. decreased during standing in 21 out of 24 experiments; 2, the $CO_2$ output fell in 12 out of 24 experiments, and 3, the ventilation volume decreased during standing in 9 out of 24 experiments.

Observations on the respiratory exchanges during a reclining period following a 10 minute standing period showed that during the first minute of recovery the $O_2$ consumption may be as much as 200 per cent greater than that of the preceding control reclining period. Within three minutes the $O_2$ returned to the resting level. We conclude therefore that standing quietly results in the accumulation of an $O_2$ debt.

The $CO_2$ and $O_2$ content of venous blood taken both from the hand and from the foot while reclining and after a ten minute period of standing was determined. The $CO_2$ content of the blood taken from the foot was always greater at the end of the standing period than during reclining, while the

blood taken from the hand showed a decrease in $CO_2$ content as a result of standing.

These results suggest that during standing there is a tendency for the blood in the lower extremities to stagnate. This results in a partial withdrawal of an appreciable quantity of blood from the pulmonary circulation. Therefore the blood in the upper part of the body passes through the lungs more frequently and is in a sense overventilated even though the total pulmonary ventilation may be decreased. The circulatory adaptations rather than changes in respiratory activity are, therefore, the primary cause of the drop in alveolar $CO_2$ as well as of the $O_2$ debt and the changes in blood gases that result from the erect posture.

*Brain metabolism and brain wave frequencies.* HUDSON HOAGLAND. Clark University and the Worcester State Hospital, Worcester, Mass.

We have found (1) that human alpha frequencies follow the Arrhenius equation as a function of temperature when subjects are given diathermy, yielding temperature characteristics of circa 8,000 calories for normals and mildly affected general paretics; circa 11,000 for intermediate paretics, and circa 16,000 for advanced paretics. These values coincide with 3 of the 6 major temperature characteristic groupings for 76 determinations of cellular respiration found in the literature. Evidence indicates that the value of circa 16,000 represents the energy of activation of a pacemaker reaction (slowest step) corresponding to an iron catalysed link in the respiratory serial chain of chemical events in cellular respiration. In general paretics, depending on the extent of infection, spicules of Fe are found precipitated in the cortex. From a reexamination of analyses of brain Fe reported by W. Freeman, this Fe results from destruction of Fe containing complexes present in the brain and does not come from the blood. If these complexes were initially catalytic Fe (indophenol oxidase, cytochrome?), the catalytic Fe would be reduced in amount and the Fe link would be slowed and might become the pacemaker for cellular respiration yielding $\mu$ = circa 16,000 calories, as occurs in advanced paretics. (2) Hoagland, Rubin and Cameron found alpha frequencies follow, with a lag, blood sugar level. The lag is of the same order as that expected between brain sugar and blood sugar, i.e., the alpha frequency depends upon brain sugar. Sugar is the brain's primary and probably only fuel. (3) Decrease in oxygen tension decreases the alpha frequency. (4) Thyroxin augments the alpha frequency and (5) we have also found dinitrophenol (a metabolic stimulant) to augment it.

These findings are consistent with the view that brain wave frequencies (like "spontaneous" myogenic frequencies) are of the nature of "relaxation oscillations" depending on (a) the rate of building up by cell respiration of critical discharge potentials and (b) on the electrical constants (impedance) of the system. Apparently metabolic stimulants and inhibitors operate primarily through (a), thus enabling one to regard *relative* changes in brain wave frequencies under standardized conditions as measures of local brain cell respiration.

*The effect of sympathectomy on the running ability of cats.* ROBERT HODES (introduced by W. B. Cannon). Harvard Medical School, Boston, Mass.

Complete extirpation of the sympathetic ganglia and removal of one adrenal gland does not reduce the ability of dogs to perform severe exercise after the effects of the operation have worn off and a period of re-training completed. The present work deals with a similar study of cats.

Five cats which ran well in a rotary treadmill after several trials, the amount of work varying with the animals, were sympathectomized and one adrenal was removed in all but one. After the cats had recovered from the operation and had regained their weight, they were again tested.

None of these animals was able to run as long or as fast as before, and a smaller amount of work invariably left them more exhausted as judged by their refusal to stand and by deeper respiration. This limitation was noticeable as long as six weeks after sympathectomy when they were apparently quite well again. One cat which could not run effectively after sympathectomy performed much better several months later when there was evidence of regrowth of sympathetic fibers, probably to the adrenal. In two cats the subcutaneous injection of adrenine had a markedly beneficial effect in increasing the ability to work and in leaving the animals much less exhausted than when less work was done before the injection. This improvement was not produced by atropine. The effects of sympathetic regrowth and adrenine administration indicate the importance of the sympathico-adrenal system in maintaining the ability of the cat to perform severe muscular exercise.

Similar to results in the sympathectomized dog, excitement or exercise produced cardiac acceleration even after sufficient atropine was injected to paralyze the vagal cardio-inhibitors. This may have been due to cardio-accelerators in the vagus trunk, since cutting the right vagus below the recurrent laryngeal nerve and the left in the neck several days previously abolished the acceleration. When an acute experiment was done under urethane anesthesia, clamping of the carotid artery or strong stimulation of the central end of the sciatic nerve caused no cardiac acceleration.

*A subliminal response in crustacean nerve.* A. L. HODGKIN (introduced by H. S. Gasser). Laboratories of The Rockefeller Institute for Medical Research, New York City.

Experiments with isolated crustacean nerve fibers show that subthreshold stimuli produce two quite distinct effects. If the stimulus is anodal or is well below threshold, it produces a polarization potential which is not unlike that in a nerve trunk. Thus, if a brief shock is applied, the potential at the stimulating electrode rises abruptly during the shock and then declines exponentially with a time constant of about 200 $\mu$ sec. As long as the shock is below 50 per cent threshold strength, the potentials have this simple time course and are directly proportional to the stimulus. However, as soon as the shock is brought near threshold, the cathodal potential changes in a striking and characteristic way. In the first place its duration is greatly increased, and in the second it has a humped contour which is quite unlike that of the corresponding anodal polarization. It is suggested that these changes are due to a local response in the cathodal region.

The distinction between the local response and polarization potential is brought out by studying their behavior in the different parts of the recovery curve. Thus the ability to produce a local response is greatly

reduced in the relatively refractory period, so that a shock which normally evokes a large local response may produce only polarization when applied to refractory nerve. During the supernormal phase the ability to produce a local response is increased, whereas the polarization potential is unaffected or slightly reduced.

Another distinction between the two processes is that the local response spreads much further along the nerve fiber than the polarization potential. Thus in a typical experiment the polarization potential declined to $\frac{1}{2}$ of its value at 0.4 mm. from the stimulating electrode, whereas the local response fell to only $\frac{4}{5}$ of its value in that distance.

There is no reason to believe that the isolated fibers were in an abnormal or injured state, for their action potentials often exceeded 50 millivolts and they could transmit a train of impulses at 500 to 600 per second.

*A contrast of ether and nembutal as anesthetics in the cat method of assaying digitalis.* HARALD G. O. HOLCK and EDWIN L. SMITH (by invitation), Department of Physiology and Pharmacology, College of Pharmacy. University of Nebraska, Lincoln.

The cat is significantly more sensitive to digitalis under ether anesthesia than under that of nembutal (pentobarbital-sodium). Results with nembutal were the same with and without a tracheal cannula. We have further verified that cats with pulmonary complications are much more sensitive to digitalis than are normal cats; to both diseased and normal cats nembutal was given.

The estimated digitalis LD 50 in frogs was 13 per cent lower under ether, but 20 per cent higher with nembutal anesthesia as compared with normal frogs. However, rats under nembutal appeared more sensitive to ouabain than their normal controls. Female rats were more resistant to ouabain than males.

*The time and height relations of twitches following a tetanus.* R. G. HORTON (by invitation) and H. E. HOFF. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Following a tetanus, twitch heights may be greater or less than before tetanus (This Journal **119:** 463, 1937). The present study concerns the relation of contraction and relaxation times to twitch heights of skeletal muscles of frogs and spinal decerebrate cats of which the ligated or sectioned motor nerve was stimulated. The recording apparatus was a frictionless optical isometric myograph of the Sherrington type. Contractions occurring later than 10–15 seconds after tetanus show contraction and relaxation times varying approximately *pari passu* with the height. Immediately following tetanus, twitch times rarely vary in proportion to the height but are in many cases proportionately much greater. Residual effects of a tetanus, apart from those of fatigue, may be noted up to at least one-half hours after tetanus.

The following is a record from tibialis anticus after 5 hours of stimulation at an average rate of about 80/min. The previous tetanus was $1\frac{1}{2}$ hours before this record. The twitch rate for this record was about 138/min. and the tetanic rate 90/sec. for 6.8 sec. 10 units of height equal about 125 grams.

| TWITCHES | UNITS INITIAL TENSION | CONTRACTION HEIGHT UNITS ABOVE INITIAL TENSION | CONTRACT TIME SIGMA | RELAXATION TIME SIGMA | COMMENT |
|---|---|---|---|---|---|
| Before | 4.7 | 20.7 | 27 | 31.1 | |
| After | | | | | |
| 1 | 9 | 23 | 31.4 | 38.7 | |
| 2 | 7.4 | 25.7 | 31.7 | 41.6 | Maximum height and relaxation |
| 3 | 7.1 | 25.2 | 32 | 40.5 | Maximum contraction time |
| 5 | 7 | 24.3 | 31.1 | 38.5 | |
| 7 | 7 | 23.7 | 30.6 | 38.3 | |
| 9 | 6.9 | 23.5 | 30.2 | 37.6 | |
| 12 | 6.7 | 23.6 | 30.6 | 38.0 | |
| 20 | 6.4 | 24.4 | 31 | 40.2 | |
| 40 | 6.3 | 24.7 | 31 | 41.6 | Orbeli effect (?) |
| 75 | 6.1 | 24.3 | 29.6 | 40 | |
| 150 | 5.7 | 23.4 | 29 | 38.7 | |
| 300 | 5.4 | 22.1 | 27.2 | 35.6 | |

*A temporary phase of extra-testicular andromimetic hormone production in rodents, associated with the adrenal X zone.* EVELYN HOWARD. Department of Physiology, The Johns Hopkins University School of Medicine, Baltimore, Md.

The reactions of the prostate and seminal vesicles following castration, in rats and mice, show that an extra-testicular factor produces considerable epithelial stimulation in male accessories during a portion of the animal's lifetime. Castrations were performed at various ages from birth to 400 days and the accessories studied after post-operative intervals of 10 to 200 days. Castrations performed at birth are followed by marked differentiation and stimulation of accessories in both rats and mice. In rats, the stimulation (Price effect) is confined to the prostate and is of brief duration, and, as described by others, castrations at 30 days of age or later result in uniform and marked degeneration of accessories. In mice, however, the stimulating effect involves both prostate and seminal vesicles, and lasts for a longer proportion of the total life span. Thus mice castrated at 21 days of age show maintenance of columnar vesicle epithelium for 100 days, and only an incomplete epithelial regression after 200 days, whereas mice castrated at 50 days or over show more rapid regression. The extra-testicular factor thus goes through a period of activity which on a smaller scale and for a briefer time is analogous to the maturation, activity, and senescence of the gonads.

The adrenal cortex goes through an associated histological process which in the mouse includes the X zone differentiation and degeneration, and in the rat consists of a transitory modification in the cortex bearing some resemblances to the X zone. Since andromimetic activity has been shown to be present in cortical extracts, it seems probable, on the basis of the present observations, that the andromimetic activity of the cortex undergoes considerable variations, and that the adrenal cortex normally produces a small scale imitation of the endocrine function of the testis, both qualitatively and temporally. If the observed andromimetic activity does originate in the adrenal cortex, it is by no means confined exclusively to

completely differentiated X zone, although present data indicate that it is greater in X zone bearing adrenals than in those without X zone.

*The velocity of removal of injected thorium from the blood.* CHARLES HUGGINS and H. L. HARRINGTON (by invitation). Department of Surgery, University of Chicago.

Colloidal thorium dioxide was injected intravenously in 12 dogs at a rate of 0.250 gram per kilogram, and thorium determinations were made on the plasma at frequent intervals subsequently. This electro-negative colloid in submicroscopic particles is insoluble, is not excreted by the organism, does not pass through normal capillaries, and may be quantitatively determined. In 10 normal dogs the curve of removal was similar, but in 2 dogs removal was much faster than in the others.

The velocity of removal is not affected by an injection of thorium 24 hours previously, by splenectomy, ether or nembutal anesthesia, cooling within physiological limits, or bilateral nephrectomy with uremia. It is slowed by an injection of thorium 2 hours previously and by removal of 1 or 2 large lobes of the liver.

*Factors influencing the weight of the thymus gland of the rat.* MELIHA INAY and KENNETH W. THOMPSON (introduced by J. F. Fulton). Laboratory of Physiology and Department of Surgery, Yale University School of Medicine, New Haven, Conn.

While studying the records of many autopsied immature rats the fact was noted that the weights of the thymus glands, although apparently highly variable, could be correlated roughly with the gain in weight of the animals. Rats of the same age that gained most weight had the largest thymus glands. These animals had been fed the stock G. L. F. calf-meal diet with vitamin and salt supplement. Similar observations were then made with the feeding of a special growth-promoting diet devised by Mendel, which induced a greater gain in weight and resulted in uniformly larger thymus glands. In these conditions with special diet, age, sex, strain of rats, and room temperature controlled, a study of some factors which influence the thymus gland was possible. The factors studied and the results were as follows:

1. Gonadectomy caused marked hypertrophy of the thymus, which reached a maximum average weight of 507 mgm., fifty days after castration as compared with the average thymus weight of 322 mgm. for the control rats.

2. Gonadotropic hormone (pregnant mare serum) injection in doses as small as 1 R.U. per rat per day for a period of 6 days was followed by involution of the thymus, only when the gonads were present.

3. Estrin (30–200 I.U. per rat, per day) and testosterone (0.25–0.5 mgm. per rat, per day) administered for two weeks produced involution of the thymus in both the normal and the castrate rats. The effect was accentuated when either of the sex hormones was given simultaneously with a gonadotropic preparation.

4. Progesterone (Prolution) in doses of 0.1–0.2 I.U. per rat, per day for two weeks had no effect upon the size of the thymus.

5. Hypophysectomy caused a marked reduction in the size of the thymus, which was increased in weight in other rats by injection of 5 growth units per rat, per day for 20 days of pituitary growth hormone (Squibb).

6. After adrenalectomy the thymus was found to be large. Gonadectomized rats, which had the largest thymus glands of any group, survived adrenalectomy for a longer period than non-castrate adrenalectomized rats. Gonadectomized-thymectomized rats survived adrenalectomy for a shorter period than either of the two preceding groups.

*The excretion of antidiuretic substance by cats.* W. R. INGRAM, LAURA LADD (by invitation) and J. T. BENBOW (by invitation). Department of Anatomy, State University of Iowa, Iowa City.

Efforts have been made to determine whether or not antidiuretic substance may appear in the urine of cats, using, with some modifications, the methods of Gilman and Goodman and of Burn. The use of cat's urine presents some difficulty because of its unusual content of toxic material; this may be controlled by proper dialysis and concentration. The urine of normal cats and cats with diabetes insipidus was found to contain no antidiuretic substance, and none appeared in the urine of polyuric animals even when the latter were dehydrated practically to the point of death by withholding fluids. Normal cats require strenuous treatment to produce dehydration as indicated by blood concentration, etc., but under such conditions may produce significant quantities of antidiuretic substance. Rough assays of the amount of antidiuretic material indicate that 27 to 360 milliunits may be excreted in 24 hours (expressed in terms of the antidiuretic activity of standard quantities of Pitressin) by cats undergoing dehydration. These observations confirm those of Gilman and Goodman for other forms and suggest that after interruption of the supra-optico-hypophyseal nervous connections a mechanism for calling forth the production of increased amounts of antidiuretic substance in time of need no longer exists.

*Control of respiration in diving animals.* LAURENCE IRVING. Edward Martin Biological Laboratory, Swarthmore College, Swarthmore, Pa.

Seals, beaver, and muskrats can withstand submersion for about five times as long as land mammals. Related to their ability to survive without breathing is the insensitivity of their respiratory adjustments to $CO_2$ which is in sharp contrast to the situation in land mammals.

The measured volume of ventilation of unanesthetized seals was only irregularly increased by 10 per cent $CO_2$. Under anesthesia 10 per cent $CO_2$ increased the frequency of breathing in anesthetized seals, beaver and muskrats slightly and the amplitude more, but breathing remained relatively easy.

The blood flow through the muscles of beaver and muskrats was scarcely reduced at all by 10 per cent $CO_2$ although in land animals the reduction in flow is usually prompt and conspicuous. In the same beaver and muskrats clamping the trachea reduced muscular blood flow promptly and conspicuously.

Anesthetized diving animals do not tolerate lack of $O_2$ in the inspired air noticeably better than dogs or cats, but they will survive clamping the trachea much longer.

The anomalous sensitivity of the respiratory adjustments of diving animals suggests that the respiratory stimuli have a different order of effectiveness in land animals and divers. The different emphasis upon the system of control may furnish a useful view of the control of mammalian respiration.

*Some simple methods for the identification of the blood of man and certain other mammals.* M. H. JACOBS, H. N. GLASSMAN (by invitation), E. P. LAUG (by invitation) and A. K. PARPART. Department of Physiology, University of Pennsylvania and Department of Biology, Princeton University, Princeton, N. J.

The observations to be reported suggest that the erythrocytes of man and some 15 other species of mammals can readily be identified by certain hemolytic reactions depending for the most part on their relative permeabilities to a series of penetrating but otherwise harmless solutes. The most useful single substance for this purpose is glycerol, which in solutions approximately isosmotic with blood, by the methods here employed, gives characteristic times of hemolysis for the different species in question ranging from a few seconds for the groundhog to approximately 40 minutes for the ox.

In addition to differences in absolute permeability to glycerol the various species also show characteristic and apparently constant differences in the manner in which this permeability is modified, in the presence of an appropriate buffer, by changing the pH of the medium. Not only is the direction and the magnitude of the effect of increasing acidity different for different species, but the pH values at which in certain species retardation of hemolysis first appears seem likewise to be specific characters of considerable constancy. Other striking specific differences are found in connection with differences in the mode of action of traces of copper and mercury on the rate of glycerol hemolysis in solutions of controlled pH, and in the effects of certain alcohols on the same process.

Other non-electrolytes which have proved to be of value for bringing out specific differences are mannitol, erythritol, xylose, thiourea and monoacetin. Among the electrolytes several ammonium salts, particularly the borate, and the chloride in the presence of low concentrations of the benzoate, enable further distinctions to be made. In the case of a few species differences in the rates of acid and alkaline hemolysis and in osmotic behavior are also useful as supplementary specific characters.

*Variations in blood pressure with skeletal muscle tension (action-potentials) in man. II. The heart beat.* EDMUND JACOBSON. Laboratory for Clinical Physiology, Chicago.

In previous studies (Am. J. Physiol., 1936; Annals Int. Med., in press) systolic and diastolic pressure were measured in man, lying at rest, at intervals beginning about fifteen minutes after reclining, while potentials were recorded (in microvolts within the frequency range, 30–4000) from electrodes in one or more of the following groups: the flexor muscles in the right upper arm, the left quadriceps femoris muscles, the abdominal muscles, muscles in the region of the right eye. Apart from fall of pressure attributable to the recumbent position, blood pressure seemed to vary with skeletal muscle tension. It was indicated that the ratio of decrement of pressure to decrement of muscular voltages, as recorded, was often greatest when the muscular voltages dwindled toward zero ($V_m \doteq 0$).

On many of the myograms made at the same time as the blood pressure readings, small electrical records from the heart can be clearly distinguished, permitting the heart rate and the heart voltage there to be determined. In a particular individual during a specific experimental period, we can follow the variations of force of the heart beat by measuring the heart voltages at corresponding instants.

In 29 records it is readily possible to distinguish the electrocardiograms. In every instance where both systolic and diastolic pressure fall (18 out of 29), there occurs a fall also in the heart rate or in heart voltage or in both (10 out of 18). A statistical analysis indicates clearly that these results are not due to chance variation. Obviously, decline in the strength of the beat (voltage), or decrease in the rate while the strength remains approximately the same indicates relaxation, mometary or sustained, in the cardiac neuromusculature. If such relative relaxation in the cardiac neuromusculature accompanies skeletal muscle relaxation, (in addition to that which follows upon reclining), it evidently must be taken into account in explaining the observed fall of blood pressure.

*The components of the electrical response from the eye of the grasshopper.* THEODORE L. JAHN and FREDERICK CRESCITELLI (introduced by J. H. Bodine). Zoological Laboratory, State University of Iowa, Iowa City. (Read by title.)

The electrical potentials obtained from the intact compound eye of the grasshopper (Melanoplus differentialis) have been studied using single light stimuli of varying duration and intensity, and repetitive stimuli of varying frequency, duration and intensity. With single stimuli of relatively short duration (less than 0.1 second) the electrical response consists of two apparent components: an initial rapid negative spike (the A wave) followed by a slow negative component (the B wave) which greatly outlasts the period of stimulation. The relative magnitude of these two components is dependent on the condition of light adaptation of the eye. In a completely dark adapted eye the B wave is as large or larger than the A wave. This usually results in such a fusion of the two components that the initial spike is effectively masked. Light adaptation brings about a decrease in the magnitude of the B wave while affecting the magnitude of the spike to a lesser extent. As a consequence the two components of the electrical response may be dissociated in varying degrees according to the length of the light adaptation period. Through sufficient light adaptation it is even possible to obtain responses which consist only of spikes. All stages from the fused compound response to the single spike component have been demonstrated by means of repetitive stimulation.

A third negative component of the electrical response becomes definitely apparent when relatively longer stimuli (0.1 second or longer) are employed. This third element, which may be termed the C wave, and which is spike-like in character, occurs either at the moment light stimulation ceases or shortly thereafter. The variation in position of the C wave is apparently associated with the condition of light adaptation of the eye. This variability in position may merely be a reflection of the alteration in the magnitude of the B wave with light adaptation rather than a fundamental change in the process which determines the C wave.

*The contribution of the auricles to ventricular filling during complete heart block.*[1] KENNETH JOCHIM (introduced by L. N. Katz). Cardiovascular Dept., Michael Reese Hospital, Chicago.

The relative contribution to ventricular filling of auricular beats occurring at various times during the heart cycle was studied in complete heart block produced by clamping the auriculo-ventricular bundle. This was

[1] Aided by the Emil and Fanny Wedeles Fund for Cardiac Research.

analyzed from ventricular volume and aortic pressure curves simultaneously recorded on photographic paper. The volume curves were analyzed to determine the true contribution of each auricular beat, which effectively adds to the filling of the ventricles, as distinguished from the total systolic ejection volume of the auricles. It is pointed out that, of the total amount of blood expelled into the ventricles by the auricles in each beat, a certain quantity regurgitates again into the auricles during their diastole, and that this amount of regurgitation is decreased if ventricular systole begins soon enough after the beginning of an auricular systole. When the effective auricular contribution is correlated with the $A_s$–$V_s$ interval, it is found that the maximum contribution occurs when this interval is equal to about half the duration of the auricular wave on the volume curve, so that ventricular systole begins before auricular diastole, thus preventing the regurgitation of blood into the auricles. In this portion of the curve, where the $A_s$–$V_s$ interval is equal to or shorter than the duration of the auricular beat, small changes in the length of the interval produce significant effects on the auricular contribution, whereas if the auricular beats occur during other parts of the heart cycle, small changes in the interval have no significant effect. It is possible that in the normal heart, the same changes in relative auricular contribution occur with changes in the $A_s$–$V_s$ interval.

*Luminescence and respiration in relation to osmotic pressure.* FRANK H. JOHNSON (by invitation) and E. NEWTON HARVEY. Physiological Laboratory, Princeton Unive sity, and the Marine Biological Laboratory, Woods Hole, Mass.

Osmotic pressure differences across the relatively rigid cell surface of marine luminous bacteria cause rupture and cytolysis, with complete loss of luminescence, respiration and viability, only in very dilute (below 10 per cent) sea water. Respiration is not affected down to 50 per cent sea water, and viability is only slightly reduced in 15 per cent sea water. Luminescence, however, decreases in proportion to dilution, and over a wide range this effect is reversible with restoration of normal concentration.

Reversibility and viability show that the decrease in luminescence in moderately diluted sea water is not due to complete cytolysis of a few cells, but to specific effects on all cells. Dilutions of isotonic sucrose result in the same per cent changes as dilutions of sea water. Consequently, the changes in luminescence are primarily osmotic rather than salt effects, and are probably due to pressure gradients across the cell surface. Hydrostatic pressures up to 68 atmospheres, which would not affect the pressure gradient, have no effect on luminescence of bacteria in sea water or 40 per cent sea water.

Concentrated sea water, in which decrease of cell volume takes place, causes a decrease in respiration, in proportion to concentration, while luminescence is not so readily affected. Above 200 per cent sea water luminescence falls off rapidly with increase in concentration, but both luminescence and respiration are reversible up to 300 per cent sea water. In 400 per cent sea water, luminescence, respiration and viability are practically abolished.

Although NaCl has the same action as sea water, other salts in isotonic concentration exert specific effects independently on the processes of luminescence and respiration.

*The urinary excretion of vitamin $B_1$; its dependence on dietary intake in normal subjects.* NORMAN JOLLIFFE and ROBERT GOODHART (by invitation). Department of Medicine, New York University College of Medicine, and the Psychiatric Medical Service of the Third (New York University) Medical Division, Bellevue Hospital.

To determine the relationship between dietary intake and urinary excretion of vitamin $B_1$ in normal subjects, a volunteer group of resident physicians was maintained with diets of known content of vitamin $B_1$, and followed during three periods: a control period, a deficiency period, and a recovery period.

The vitamin $B_1$ deficient diet used in this study contained 382 grams carbohydrate, 79 grams protein, 144 grams fat, and 3,182 calories. Included in this diet was 6 cc. of 0.1 N. hydrochloric acid, 50 mgm. of cevitamic acid, 0.2 cc. of plain halibut liver oil (8,500 U. S. P. units of vitamin A and 145 U. S. P. units of vitamin D), liver residue (Lederle) obtained from 480 grams of whole liver, and 0.2 gram of ferrous sulphate. We believe that this diet is adequate in all respects except in vitamin $B_1$. The vitamin $B_1$ content was calculated from Cowgill's tables to be 3,155 mgm. equivalent (158 international units, or 474 micrograms of crystalline substance), which gives a vitamin/calory ratio of 0.99. This ratio, on the basis of Cowgill's prediction formula, supplies only 50 per cent of the predicted vitamin $B_1$ requirement of a subject weighing 70 kgm.

During the control period our subjects consumed either the vitamin $B_1$ deficient diet plus synthetic crystalline vitamin $B_1$, or an adequate weighed diet in which the vitamin $B_1$ content was estimated. During the deficiency period all subjects received only the vitamin $B_1$ deficient diet. This was continued for varying periods of time depending upon individual indications. In the recovery period this diet was supplemented by synthetic crystalline vitamin $B_1$. During all three periods the excretion of vitamin $B_1$ (thiamine) in the urine was determined by Cline's modification of Goudsmit and Westenbrink's chemical method. At frequent intervals observations were made as to signs and symptoms, blood chemistry, hematology, and cardiac changes as revealed by the electrocardiogram and roentgenogram.

Our studies indicate a good correlation between urinary excretion and dietary intake of vitamin $B_1$. We have also noted a correlation between urinary excretion and symptoms.

*The effect of sodium hexametaphosphate on the regeneration of epithelial tissue.* K. K. JONES, R. W. VANCE (by invitation) and QUIN DE MARSH (by invitation). Northwestern University Medical School, Chicago, Ill.

Sodium hexametaphosphate forms a film with tissue exudates that is moist, firm, flexible, and bacteriostatic. Tests with bacteria show that bacteria can live in a dilute solution, converting the $PO_3$ radical to the $PO_4$ radical which can be utilized. In concentrations of 10 per cent or more bacteria do not grow in the metaphosphate solutions although they may survive. Islets of living epithelial tissue are not injured by metaphosphate application as is shown by experiments on the dog.

Infection of burns and denuded surfaces of the skin is prevented by the application of a metaphosphate solution. Under the film, produced by the metaphosphate, epithelium regenerates over denuded areas. This

epithelium is normal in appearance. Underlying this is a highly vascular and reticulated stroma quite different from the atrophic tissue which regenerates under tannic acid films.

This will be illustrated by motion pictures and lantern slides.

*Dark adaptation and intake of vitamin A.* FREDERIC T. JUNG and BERTHA L. ISAACS (by invitation). Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

More than 400 sets of observations were made on approximately 140 subjects between the ages of 20 and 45 by using an apparatus which measures the rate of the subject's adaptation to darkness. The technique of the individual observation was like that of Jeans, Blanchard, and Zentmire, excepting that in the beginning of our study many additional readings were made; these were discontinued when the constancy of the results became apparent. From the readings there was calculated for each subject a number which may be called his biophotometric index. The indexes obtained ranged from 125 to 241. A high index means that the subject, after exposure to a light of definite brightness for a definite length of time, rapidly regained his ability to see dim lights.

Each subject also made notes on his diet over a 5-day interval, and reported his results by filling out a questionnaire. On this basis his intake of vitamin A was estimated in international units per day. The values ranged from 1650 to 9725; the method of estimation was such that 1650, for instance, meant that the subject took *al least* that many units per day.

When the biophotometric indexes were spot-diagrammed against the estimated vitamin A intake, the distribution did not suggest any relation between the two. The correlation coefficient for the data on the first 41 subjects proved to be $-0.05$. If the ability of adult human subjects to adapt to darkness is at all related to the intake of vitamin A, the demonstration of such relationship would seem to require much more elaborate methods than those used by us, or others who have reported observations in this field. The greatest source of error seemed to be the calculation of the vitamin A content in assorted diets.

*Spontaneous heart failure in the isolated heart.*[1] L. N. KATZ and M. MENDLOWITZ[2] (by invitation). Cardiovascular Department, Michael Reese Hospital, Chicago, Ill.

Recently, we made a study of spontaneous heart failure in an isolated heart circuit in which the work, oxygen consumption and efficiency of the isolated heart could be measured with minimal error. In this single circuit defibrinated and heparinified blood enters the left heart from a reservoir under a constant pressure head via the left auricle. It is pumped by the left ventricle through an artificial resistance into the right auricle and then by the right ventricle through an artificial resistance into an aerator from which it is forced back into the original reservoir by an artificial pump. When the circuit is established, adjustments are made to obtain the desired levels for blood flow and pressures. No further readjustments are made except in the pulmonary and aortic resistances, which are varied so as to keep the aortic and pulmonary arterial pressures as constant as possible.

[1] Aided by the A. D. Nast Fund for Cardiac Research.

[2] Working under a grant from the Emanuel Libman Fellowship Fund, Mt. Sinai Hospital, New York.

During the experiment, measurements are made of the pressures in the systemic and pulmonary arteries and veins as well as of the pulmonic and aortic blood flow, and of the A–V $O_2$ differences. From this data calculations are made of the coronary flow, work, $O_2$ consumption and mechanical efficiency.

It was found that in this preparation the heart failed spontaneously; usually the left ventricle failing before the right. Failure of the heart was accompanied by a decrease in work and $O_2$ consumption, the mechanical efficiency remaining unchanged except for a terminal decrease. Failure of a heart chamber, this data shows, is due to a loss of contractile power so that the chamber becomes unable to do the work imposed upon it by the load. The loss of contractile power is manifested by a partial failure to release energy as indicated by a decrease in oxygen consumption and not by a change in the mechanical efficiency with which this energy is utilized. It is apparent from these results that it is a loss of ability to release energy and not a loss of mechanical efficiency which is the essence of heart failure.

*Conditioned responses to peripherally acting drugs (histamine).* S. KATZENELBOGEN (by invitation), ROGER BROWN LOUCKS (by invitation) and W. HORSLEY GANTT. Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore, Md.

The widespread vague and often ambiguous use of the concept "conditioned reflex" and its application in many unjustified instances necessitates an attempt to delimit processes which may actually be produced by the adventitious elements of the environment from processes which are independent of these elements. Previous work has shown that the effect of drugs which have a central action such as morphine may become readily conditioned. This and claims of a conditioned immunity, etc., have led to the unwarranted belief that almost any process regardless of the nature of its stimulus—acting on the central or peripheral nervous system or endings—may also become conditioned, i.e., result from the milieu in which it is given as well as from the natural unconditioned stimulus. We chose certain drugs having a definite peripheral action (independent of the central nervous system), and whose effects could be quantitatively measured, such as the production of hyperglycemia to the injection of adrenalin (Gantt, Katzenelbogen, Loucks. "An Attempt to Condition Adrenalin Hyperglycemia." Bull. Johns Hopkins Hosp. **60:** 400, 1937), and the provocation of hypersecretion of gastric juice by histamine. The injections of histamine were accompanied by definite signals ordinarily used to produce conditioned reflexes. After repeated and prolonged injections there was no sign of a conditioned gastric secretion. Although these effects of the peripheral action of the drugs (hyperglycemia and gastric secretion) could not be conditioned certain phenomena resulting from central action became conditioned.

We conclude that the effects of drugs having an essentially peripheral action independent of the central nervous system are incapable of becoming conditioned responses.

*The effect of the adrenal cortical hormone on the liver and muscle glycogen of normal fasting mice and rats.*[1] B. KATZIN (by invitation) and C. N. H.

[1] This work was materially assisted by a grant from the Endocrine Committee of the National Research Council.

LONG. Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.

Britton and Silvette (Am. J. Physiol. **100:** 693, 1932) have reported that the injection of adrenal cortical extracts into both normal and adrenalectomised fasted animals is followed by hyperglycemia and considerable increases in muscle and liver glycogen. Since these results suggest a direct effect of this hormone on carbohydrate metabolism, we have repeated them with fasted mice and rats injecting, intraperitoneally, both Upjohn's Cortical Extract and the crystalline compounds A and B isolated from the adrenal cortex and kindly supplied to us by Dr. E. C. Kendall. The results are in the table and demonstrate that a marked increase in liver glycogen is obtained with both the extracts and crystalline hormones. There is a questionable increase in muscle glycogen and a slight hyperglycemia has only been observed in a few animals.

Of great interest is the fact that the marked depletion of the carbohydrate stores of hypophysectomised rats, induced by fasting, may be completely prevented by the injection of adequate amounts of either cortical extract or compound B.

The nitrogen excretion of fasted normal rats may also be increased by suitable injections of this cortical extract.

TABLE 1

| | NUMBER OF ANIMALS | TOTAL FAST | PERIOD OF INJECTION | GLYCOGEN | | NOTES |
|---|---|---|---|---|---|---|
| | | | | Muscle | Liver | |
| | | *hours* | *hours* | *grams per 100 grams* | *grams per 100 grams* | |
| (A) Mice | 8 | 24 | | 0.236 | 0.31 | |
| | 9 | | | 0.429 | 2.65 | Fed animals |
| | 8 | | 14 | 1.037 | 9.90 | Fed animals 0.25 cc. cortical extract every hour for 14 hours |
| | 7 | 24 | 12 | 0.304 | 4.08 | 0.25 cc. cortical extract every hour for 10 hours |
| | 2 | 22 | 10 | 0.326 | 3.50 | 0.1 mgm. compound A every hour for 10 hours |
| | 4 | 24 | 12 | 0.256 | 2.75 | 0.06 mgm. compound B every hour for 10 hours |
| (B) Rats | 13 | 24 | | 0.521 | 0.19 | |
| | 9 | 32 | 8 | 0.530 | 0.77 | 1 cc. cortical extract every hour for 8 hours |
| | 9 | 36 | 12 | 0.520 | 1.08 | 1 cc. cortical extract every hour for 8 hours |

*A comparison of the effects of water, certain sodium and potassium salts on renal excretion.* NORMAN M. KEITH and ARNOLD E. OSTERBERG (by invitation). Mayo Clinic, Rochester, Minn.

Considerable information has already been obtained in regard to the action of water and different salts on renal function. It seemed, however, important to have further comparative studies in the normal individual.

Voided specimens of urine were collected half hourly or hourly for a five hour period in the morning. No food was eaten, but a total of 400 cc. of water was ingested at regular intervals over this period when a given salt was taken; while 1500 cc. of water was ingested in 15 to 30 minutes when the effect of water alone was studied. Venous blood samples were

withdrawn at repeated intervals. Control observations were made at the end of the first hour in each experiment and in 3 individuals throughout the five hour period. Sodium bicarbonate and chloride were ingested or injected intravenously in doses of 0.08 to 0.14 gram per kgm. of body weight in 5 to 8 per cent solution. Potassium salts ingested were the bicarbonate, chloride and nitrate in amounts of 0.11 to 0.16 gram per kilogram of body weight in a 25 per cent solution.

The effects produced by sodium salts were variable. In one individual taking sodium chloride the results could scarcely be distinguished from the controls. Sodium bicarbonate produced an inconstant increase in water, a decided rise in pH and bicarbonate, and some increase in phosphate and potassium. Potassium salts, however, uniformly caused a striking increase in water, pH, chloride, bicarbonate, sodium, and potassium but a decrease in phosphate. A large ingestion of water caused much the greatest diuresis and an increase of urea; changes in the other constituents were small and variable.

Alterations of the blood plasma and serum were relatively small. Consistent variations were a small increase in bicarbonate and sodium after taking sodium bicarbonate and in serum potassium after each potassium salt.

Sodium and potassium have some distinctly different functions in general metabolism and also in the kidney, where the site of action appears to be the tubular cells.

*Atonia following transection of the brainstem through the upper level of the pons.* A. D. KELLER. Department of Physiology and Pharmacology, University of Alabama School of Medicine. (Read by title.)

Complete muscular atonia and profound hyporeflexia have been repeatedly encountered in cats and dogs after transection of the brainstem through the upper level of the pons. The preparations similate in these respects the states of deep anesthesia and spinal shock.

On the basis of current teaching one would expect to encounter this state only when the transection passed through or caudal to the vestibular nuclei. Accordingly the question is raised as to whether our transections defunctioned the more or less distant vestibular nuclei. It is practically certain that this is not the case for the following reasons: (1) the syndrome follows immediately clean transections which are not complicated by hemorrhage or detectable disturbance in adjacent blood supply, (2) hypotonia does not follow extensive bilateral lesions placed at the same or lower level of the pons (i.e., nearer to the vestibular nuclei) as long as the medial fourth of the pons tissue remains unsevered, (3) the syndrome does result when a transection is incomplete laterally on both sides, thus leaving all surface vessels undisturbed, and (4) the syndrome has persisted essentially unaltered in preparations with complete transections maintained for 10 to 18 days after operation and in one preparation such as described in (3) maintained for 7 weeks after operation.

The transections were made by traction with a slender, blunt probe, the tissue above and below the lesion path remaining in place with an intact blood supply.

Transections made by this method through the caudal extent of the diencephalon do not affect materially the postural mechanisms. Transections through the lower extent of the midbrain eliminates the righting

mechanisms leaving muscle tone and the standing reflex intact. Such preparations do not exhibit extensor rigidity in that there is no resistance to passive flexion of the limbs and the standing posture is not exaggerated.

*Elimination of the heat production mechanism with a sparing of the heat loss mechanism.* A. D. KELLER. Department of Physiology and Pharmacology, University of Alabama School of Medicine. (Read by title.)

We have previously reported procedures whereby (cat and dog) the heat production mechanism can be markedly impaired without eliminating the animal's ability to pant. Procedures: (1) Placing a wide medial transverse lesion at the upper level of the pons such that all descending fibers are severed except those passing in an extreme lateral position, (2) transection of the brainstem through the most caudal extent of the diencephalon, and (3) severing the dorso-caudal hypothalamic connections.

Is the heat loss mechanism in these preparations adequate to prevent a rise in body temperature? When such chronic preparations, one or more in each category, were subjected to cool environments their rectal temperatures fell to low levels (30°C.) without shivering. When they were subjected to a warm environment, a low rectal temperature being present at the outset, the rectal temperature rose progressively to from 38°C. to 40°C. at which time typical thermal polypnea began. With the onset of polypnea the rectal temperature fell slightly and then was maintained constant.

It is evident, therefore, that the activation of the heat loss mechanism is independent of the heat production mechanism. A certain release of the heat loss mechanism from coördinated control was evident, however, in that following a test after the environmental temperature was lowered, the heat loss mechanism continued active for some time even though the rectal temperature was lowered to below normal. This would seem to indicate the existence of a reciprocal innervation between the two mechanisms in the intact animal.

It may be that the structural elements in the cephalic brainstem subserving the heat loss and heat production mechanisms have separate loci to the extent that at least the caudal portion of the former reaches into the cephalic midbrain whereas the latter may be predominantly confined to the hypothalamus. The fibers descending from the heat loss mechanism appear to have a greater lateral distribution, at the level of the pons, than do the heat production fibers.

*The water exchange in the dog following hypophysectomy procedures.* A. D. KELLER. Department of Physiology and Pharmacology, University of Alabama School of Medicine.

The water exchange under uniform conditions of diet and following anterior pituitary extract or thyroid administration has been studied in one group of dogs in which total hypophysectomy was attempted without infringement upon the hypothalamus and in another group in which subtotal hypophysectomy was purposely performed, the hypophyseal stalk being left attached to the brain. Histological studies made after the termination of the experiments revealed that in the first group small remnants of tuberalis remained while in a few there was some encroachment upon the hypothalamus.

The dogs in which the hypophyseal stalk remained intact displayed the first day after operation a moderate polydipsia and polyuria, but on subsequent days the water exchange returned to normal or below where it was maintained. The water exchange in these preparations was resistant to anterior pituitary extract or thyroid.

The animals in which only remnants of stalk tissue remained also exhibited a moderate diabetes insipidus the first day after operation. Subsequently the water exchange in some of the animals returned to normal while in others after some reduction it leveled off above normal, i.e., a mild diabetes insipidus persisted. The water exchange in this group of animals was sensitive to anterior pituitary extract or thyroid.

*Present interpretations:* 1. The pars anterior is not essential for the occurrence of diabetes insipidus but its functional presence greatly intensifies the effects of a deficiency in the antidiuretic principle. 2. The tissue of the hypophyseal stalk or hypothalamus possesses adequate antidiuretic secretory powers to successfully regulate the water exchange in the functional presence of the pars anterior. 3. A small functional remnant of antidiuretic secretory tissue is able to regulate the water exchange under the conditions which obtain in the absence of the pars anterior but is inadequate to meet additional demands.

*Anoxemia of the kidney as a cause of uremic acidosis: Inhibitory effect of low oxygen tension on the deamination of aminoacids in kidney tissue.* WALTER KEMPNER (introduced by F. D. McCrea). Department of Medicine, Duke University, Durham, N. C.

The rate of deamination of d,l-alanine, d,l-valine, d,l-leucine in surviving intact kidney tissue and in kidney extracts was measured manometrically by the Warburg method at various oxygen tensions. The deamination rate proved markedly inhibited by oxygen tensions below 8 Vol. per cent. In intact tissue slices the deamination rate of d,l-alanine, e.g., was inhibited at 5.4 Vol. per cent oxygen by 60 per cent, in kidney cell residues at 3.4 Vol. per cent oxygen by 49 per cent as compared with the deamination rate in air. The inhibitory effect of lowered oxygen tension on deamination was found in tissue slices and extracts whether the suspension medium was phosphate or $CO_2$-bicarbonate solution. It was independent of pH changes (m/30 phosphate) between 6.8 and 8.1. The inhibition of deamination by lowered oxygen tension was entirely reversible.

These experiments show that cessation of the chemical function of the kidney is not necessarily due to anatomical destruction but can be caused by a temporary change of the gas concentration in the cell environment. Lowered oxygen tension in the kidney reduces the aminoacid deamination with corresponding ammonia formation to a fraction of the normal rate. The aminoacid deamination being the main source of urine ammonia (Krebs), renal anoxia leads to disturbance of the acid-base equilibrium in the body: Blood and tissue acids reaching the kidney as sodium salts and normally converted there into ammonia salts, are now, due to the inhibited renal ammonia production, excreted into the urine as sodium salts; the blood bicarbonate drops, uremic acidosis ensues. But since the inhibition of deamination of aminoacids by lowered oxygen tension is a reversible reaction, the original rate of ammonia production is reëstablished

with the rise of the oxygen tension to its physiological level. Transient uremic acidosis is thus explained as a result of the reversible inhibition of the deamination of aminoacids in states of renal anoxemia.

*Recovery of function in infant monkeys following cortical ablations.* MARGARET A. KENNARD. Laboratory of Physiology, Yale University School of Medicine, New Haven, Conn.

Following bilateral ablation of areas 4 and 6 of Brodmann recovery of motor function in young or adult monkeys is minimal. In contrast the infant monkey operated at three weeks regains the motor power essential for eating, walking and climbing although the fine movements of the digits never develop the delicacy of coördination of the normal animal (Am. J. Physiol. **115:** 138, 1936.) At all ages recovery is due in part to integration through remaining "non-motor" areas of the cortex. In the infant this is markedly so since subsequent ablation of the remaining parts of the frontal lobes (areas 8, 9, 10, 11 and 12), first from one side and then from the other, is followed, after each operation, by a marked reduction in adequacy of motor performance (chiefly in the contralateral extremities, but to some extent in the ipsilateral also). Motor performance then is seriously impaired and feeding by means of the hand becomes impossible although motor progression can still be executed. Similarly ablation of the postcentral regions, following removal of areas 4 and 6 in infancy, has a profound effect on motor performance.

Increase in resistance to passive manipulation is consistently present in the adult following unilateral removal of areas 4 and 6, and is extreme after bilateral ablation of these areas. In the infant, after similar operations, no increase in resistance to passive manipulation can be felt, the extremities remaining flaccid for many months. However, during the second and third years of life considerable increase in resistance to passive manipulation develops in such animals. Contractures of the tendons of the leg also tend to appear at this time.

*Pulmonary ventilation in artificial respiration.* BARRY G. KING (introduced by Walter S. Root). Department of Physiology, College of Physicians and Surgeons, Columbia University.

A comparison of the Nielsen, the Schäfer and the "combined" methods (C. K. Drinker, J. Ind. Hyg. **17:** 243, 1935) of artificial respiration has been made. The present study has been limited to the evaluation of ventilation disregarding other aspects of the methods employed. Determinations were carried out on conscious human subjects. An adequate ventilation was maintained by trained operators so that the subjects had no desire to breathe. Intelligent coöperation was obtained by selecting members of the teaching staff and first and second year medical students.

The expired air was collected in a spirometer for measurement of minute volume and $CO_2$ content. Artificial respiration was applied for a short period prior to the collection of the expired air. The minute volume was maintained essentially constant when the prone pressure was applied 14 times a minute, the Nielsen at 7 and the "combined" at 5 times a minute. At these rates the $CO_2$ content of the expired air was essentially the same for the three methods. The rates of application necessary to maintain the minute volume of the individual constant for the three methods did not vary with different operators.

It was concluded that the "combined" method may offer the definite advantage of adequate ventilation with less frequent applications of pressure. In extreme asphyxia the "combined" technique could be given at a faster rate employing the inhalator with suitable concentrations of $CO_2$.

*The effect of sulfanilamide on blood leucocytes.*[1] JOSEPH T. KING. Department of Physiology, University of Minnesota.

The influence of sulfanilamide on the migration rate of blood leucocytes has been studied in tissue cultures. Methods and equipment have been described in the Arch. f. Exper. Zellforsch (**9:** 341, 1930; **10:** 467, 1931; **20:** 208, 1937).

Fragments of buffy coat from rabbit blood were matched for size and divided into experimental and control sets. A series consists of 30–36 cultures made from one animal. Fragments were planted in one part autogenous heparinized plasma and three parts autogenous serum extract of 7 day chick embryos. Rate of migration was measured with 16 mm. objective and 6x ocular with micrometer (114 units = 1 mm.). Sulfanilamide was dissolved in 0.9 per cent NaCl at room temperature and sterilized by filtration. Cultures were planted in moist chambers (Maximow) and incubated as lying drop preparations.

Observations have been made on cultures containing sulfanilamide in concentrations between 1:1,000 and 1:1,000,000. Results on the 1:1,000 concentration only are given below for the time intervals indicated.

Values refer to average maximal width of the migration zone. Since the periphery contains few lymphoid cells, the values are taken to represent the migration rate of granulocytes.

Conclusion:

In the presence of 1:1,000 sulfanilamide the granular leucocytes of rabbit blood in tissue culture migrate at a more rapid rate than in the control cultures.

| INCUBATION TIME | NUMBER OF SERIES | AVERAGE EXPERIMENTAL | AVERAGE CONTROL | ABSOLUTE DIFFERENCE | S.E. OF DIFFERENCE |
|---|---|---|---|---|---|
| *hours* | | | | | |
| 2 | 8 | 107.83 | 93.15 | 14.69 | 2.600 |
| 4 | 9 | 160.62 | 137.10 | 23.52 | 3.977 |
| 8 | 8 | 222.31 | 190.20 | 32.11 | 3.919 |
| 24 | 7 | 326.85 | 276.41 | 50.44 | 8.614 |

*Studies on nutritional muscular dystrophy in rats.* G. C. KNOWLTON (by invitation) and H. M. HINES. Department of Physiology, State University of Iowa. (Read by title.)

Studies were made upon the chemical composition, histological and physiological characteristics of skeletal muscle from rats reared from weaning on a vitamin E-deficient diet. Female rats on the same basal diet with added vitamin E concentrates adequate for almost continuous fertility served as controls. The animals were made available by the cooperation of the Department of Biochemistry. The studies on the vitamin E-deficient rats were made on the gastrocnemius muscle at 97 to 190

[1] Aided by a grant of the Committee on Scientific Research of the American Medical Association.

days of age. The muscles showed only minor or indefinite histological changes characteristic of atrophy or dystrophy. An increased chloride and water and a decreased creatine concentration was found in the muscles. These changes in chemical composition which are characteristic of atrophy and dystrophy were more pronounced in the older than in the younger animals. The isometric tension developed by direct stimulation of the muscle was definitely below normal. Furthermore, the tension developed upon stimulation of the motor nerve was always considerably less than that elicited by direct stimulation. The muscles of these rats also showed an increased sensitivity to acetylcholine. These changes are characteristic of muscular atrophy and dystrophy, the functional and chemical changes being much more marked and definite than the histological findings.

*Experiments on auditory fatigue.* H. G. KOBRAK, J. R. LINDSAY and H. B. PERLMAN (introduced by A. J. Carlson). Division of Otolaryngology, University of Chicago, Ill.

Acoustic reflexes offer a method of objective hearing tests both in the animal and in the human. While the hearing sensation involves the peripheral sense organ as well as higher centers the acoustic reflex of the muscles of the middle ear is independent of the cortex. Complicated acoustic phenomena e.g., auditory masking or auditory fatigue can be analyzed by comparison between hearing sensation and reflex responses.

The following observations on auditory fatigue were made:

1. In the human short loud tones (2 min. of 90, 95, or 100 db.) produce and elevation of the threshold of hearing, that is specific regarding the frequency. The recovery is rapid.

2. Identical stimulation does not produce any alteration of the threshold of the tensor reflex in the rabbit.

3. Longer continued loud tones (45 min.) give increased and prolonged signs of fatigue in the human and definite signs of fatigue of the acoustic reflex in the rabbit. The "speed of recovery" in the human is different from that of the animal.

4. A comparison of the Wever and Bray phenomenon with the acoustic tensor reflex shows a great discrepancy of results. Hughson and Whitting report that electrical potentials of the acoustic nerve, after a tone of 35 minutes duration, are fatigued for 24 to 72 hours covering the whole tone range. The tensor reflex shows a complete recovery in a few minutes.

5. Interrupted tones with pauses long enough to permit relaxation of the middle ear muscles do not fatigue the tensor or stapedius.

6. In the presence of two tones of different frequencies one given continuously, the other interrupted, the continuous tone shows signs of fatigue while the interrupted tone shows no fatigue.

*Discussion.* All symptoms of fatigue as observed in these experiments are not located in the muscle itself, since other stimuli still produce muscular contractions. A definite localization of fatigue is not possible with the available evidence. However the results suggest that fatigue is not necessarily confined to one point of the auditory sense organ. The different results obtained with short and long stimuli may be explained as fatigue symptoms of different localities. Short, loud tones do not alter the sensitivity of the peripheral sense organ. Interrupted acoustic stimuli as occurring in daily life do not fatigue the acoustic tensor reflex!

*Concerning the conversion of androgens to estrogens in the animal body.* CHARLES D. KOCHAKIAN (introduced by John R. Murlin). University of Rochester, Rochester, N. Y.

In a previous report it was demonstrated that androgens injected into dogs, rabbits and human subjects, could be recovered in the urine only to the extent of 2–6 per cent. It was suggested at that time on the basis of data in the literature that some of the lost androgenic activity might be due to conversion to estrogens. This hypothesis was investigated by determining the estrogenic content of the urine of 2 castrate dogs and 1 normal dog for 2–3 periods of 4–7 days each after the injection of the following androgenic preparations: urine extracts, androstenedione (180, 60, 180 and 139.5 mgm.), testosterone (45, 60 mgm.), testosterone benzoate (50 mgm.) testosterone oxime (128 mgm.), and testosterone acetate (60 and 75 mgm.). The Allen-Doisy rat technique for observation of estrus was used. At first 6 rats were used for each period and this was reduced to 2 as the results were repeatedly negative. There was not one instance of a positive estrus smear. Therefore not more than the equivalent of 2$\gamma$ of estrone could have been excreted. At best this is an insignificant amount when compared to the amounts of androgens injected. For example, in one experiment 180 mgm. androstenedione were injected or 90,000 times more than the greatest possible amount of estrogens (as estrone) excreted.

*The effect of certain foods on bile volume output recorded in the dog by a quantitative method.* E. J. KOCOUR (by invitation) and A. C. IVY. Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Ill.

By applying continuous suction to the biliary system of bile fistula dogs consistent and reproducible results were obtained in the volume output of bile under standard controlled experimental conditions. The effect, on the volume output of bile, of the feeding of meat (protein), of olive oil (fat) and of syrup (glucose), and the effect of the intravenous administration of glucose and of fluids each with and without the return of bile will be presented.

*Gastrin.* S. A. KOMAROV. Department of Physiology, McGill University, Montreal, Canada.

Preparations having a strong secretagogue effect on the fundic glands of the stomach were isolated from the pyloric mucosa. These preparations contained no histamine, choline or other organic crystalloids, and were also devoid of any fatty material soluble in acetone, benzene and ether. Gastric secretion elicited by intravenous injection of such preparations in acute experiments on cats was characterized by high acidity and low peptic power, and was not affected by atropine, even in large doses. In its general chemical properties the secretagogue principle of pyloric extracts has much in common with secretin. It is quantitatively precipitable by 10 per cent trichloroacetic acid and by 30 per cent NaCl, and it is soluble in 80 per cent acidulated alcohol and in dilute acetone. A routine chemical procedure was developed whereby histamine-free preparations, containing both secretin and the secretagogue principle of pyloric extract could be isolated from the tissues. In this way the distribution of the pyloric principle and of secretin in different regions of the mucosa of the

digestive tract was studied. It was found with regard to the gastric secretagogue effect that the most potent extracts were those isolated from the pyloric mucosa; the extracts from the mucosa of the upper duodenum were much less active, and those from the mucosa of the lower duodenum still less so. Extracts from the resting fundic mucosa did not stimulate gastric secretion at all. As to the effect on pancreatic secretion, extracts from the duodenal mucosa were naturally very potent, but only a slight secretin-like effect on the pancreas was observed after intravenous injections of pyloric or fundic extracts. In many instances highly potent pyloric extracts were prepared, which had no secretin-like effect on pancreatic secretion. The above results appear to support the "gastrin" theory of gastric secretion. They also suggest that "gastrin" may play some part in the intestinal phase of gastric secretion.

*The osmotic function of the chicken's kidney.* IRVIN M. KORR (introduced by H. W. Smith). Department of Physiology, New York University College of Medicine, New York City.

The kidneys of chickens drinking ad lib. normally excrete urine (0.1 to 0.5 cc. per min.) varying in osmotic pressure between 0.15 per cent and 1.1 per cent NaCl (mode about 0.98 per cent) as measured by the Baldes thermoelectric v.p. method. Blood ranges in osmotic pressure from 0.925 per cent to 0.940 per cent NaCl. During water deprivation the urine osmotic pressure increases, attaining values in 2 to 3 days of 150–170 per cent that of the blood, the osmotic gradient: tubular urine blood = 0.7 per cent NaCl. Urine flows are 10 per cent of normal or less. Pitressin in the normally hydrated bird also causes the excretion of unusually hypertonic urine. Even greater differences between blood and urine (urine o.p. = 180 per cent blood o.p.; osmotic gradient = 1 per cent NaCl) are produced by hypertonic NaCl solutions intravenously.

Administration of 100 cc. of water by stomach induces dilute urine (3 cc. per min. in 3 kgm. birds). Inulin clearances show that the normally low urine flow is attended by a low filtration rate (3.5–4 cc. per min.) which, after the administration of water, is increased to high values (7–8 cc. per min.) and tends to remain relatively constant during the diuretic cycle. This suggests the possibility that during water deprivation in the bird glomerular activity can be considerably reduced.

The administration of epinephrine at low urine flow increases filtration considerably, with only small or negligible increase in urine flow.

Although the data showed that about 700 cc. of water are absorbed from the cloaca per day (apparently by the rectum), the osmotic relations of ureteral urine and cloacal fluid indicate that this absorption takes place isosmotically, due to the simultaneous reabsorption of chloride.

The statement, often found in the literature, that the avian kidney is incapable of producing hypertonic urine, and that if there is any indication of hypertonicity in the bird's excreta it is attributable to the cloaca, is therefore shown to be fallacious.

Crane, Marshall and others have suggested that osmotic reabsorption in mammals is done in the thin segment of the loop of Henle. Since some nephrons in the chicken have an apparently homologous segment, the demonstration that the chicken kidney can elaborate hypertonic urine lends support to this suggestion.

*Oxidation-reduction potentials in heterogeneous systems.* IRVIN M. KORR (introduced by E. Newton Harvey). Physiological Laboratory, Princeton University, Princeton, N. J. (Read by title.)

The reactions and oxidation-reduction potentials, in homogeneous solutions, of many substances which function within the cell as "hydrogen carriers" or oxidative catalysts, have been extensively investigated in the last decade. In applying these data to the interpretation of the intracellular functions of these systems, the fact that their behavior may be grossly affected by the presence of interfaces, such as exist in a polyphasic mixture like protoplasm, has not always been considered.

An exploratory investigation of the behavior of five redox indicators, including the bacterial pigment, pyocyanine, in simple but heterogeneous systems (suspensions of cellulose, kaolin, silica and alumina gels), has brought to light a number of facts which must be taken into consideration for a fuller understanding of biological redox potentials and of the function of hydrogen carriers:

1. In a heterogeneous system, the potential measured at an electrode may be very different from that corresponding to the ratio of oxidant to reductant present in the whole system, due to differential adsorption. The potential at the interface, therefore, may be vastly different from that of the continuous phase.

2. These reversible systems can act as carriers of hydrogen from surface to surface, by being reduced at one and reoxidized at another. This mechanism may also be of importance in the transfer of energy from surfaces at which energy-producing oxidations are occurring to those at which energy is needed, e.g., syntheses.

3. The rates of reaction of these systems are profoundly modified by adsorption.

4. In a system like pyocyanine, under conditions in which one-step (bivalent) oxidation-reductions predominate in solution, it is possible for two-step (univalent) processes to predominate at interfaces between that same solution and other phases. A similar mechanism in the cell would ensure the existence of adequate concentrations of the intermediate (semiquinone) forms at strategic surfaces (e.g., those of dehydrogenases), despite the minimal proportion of total (and already very dilute) carrier which appear in this form at the intracellular pH. This would reconcile the extremely unphysiological pH ranges in which two-step oxidations predominate with their apparently great importance (Michaelis, Shaffer) in biological oxidations.

*Some effects of anti-thymus serum on rats.* C. P. KRAATZ (introduced by L. B. Nice). University of Cincinnati and Department of Physiology and Pharmacology, Chicago Medical School, Chicago, Ill.

During a period of 26 months, a male goat was injected subcutaneously with suspensions of strained material representing the thymus glands of 1158 rats 6 to 10 weeks of age. Successive bleedings provided an anti-thymus serum which tended to decrease in potency and specificity. Control sera were obtained from two normal male goats and one male goat injected for 6 months with a total of 95 cc. of strained rat liver substance.

The effects of single intraperitoneal injections of anti-thymus serum

into rats were: loss in body weight, an initial drop in the white blood cell count followed by a rise above the normal level, a decrease in the red cell count appearing 24 to 48 hours after the injection and persisting at a low level for 5 days, enlargement of the adrenals and kidneys, shrinkage of the thymus, frequent shrinkage of the spleen, and with sufficient dosage severe anemia and death.

Daily intraperitoneal injection of small doses of anti-thymus serum evoked: no change in body weight, a high white blood cell count with an increased percentage of eosinophil and neutrophil polymorphonuclear leucocytes, a low red cell count with macrocythemia and polychromatophilia, a great enlargement of the spleen with myeloid metaplasia, and maintenance of thymic size. After cessation of injections the blood picture returned to normal.

Anti-liver serum in large doses reduced the red cell count slightly. Other control sera administered in twice the dosage of anti-thymus serum had no similar effect.

Anti-thymus serum in single or repeated injections had no effect on the onset or sexual maturity in female rats or their subsequent reproductive activity.

*Proliferation of crop sac epithelium in incubating and in prolactin-injected pigeons studied with the colchicine method.* ERNEST L. LAHR (by invitation) and OSCAR RIDDLE. Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

The number of mitoses arrested by colchicine in cells of the crop sac epithelium of adult pigeons was used as a measure of prolactin-induced proliferation. It was first shown that the optimum dose of colchicine is 0.6 mgm. (in 400–500 gram birds) when this is injected intramuscularly in a single dose; also that the full effect of the dose is not attained in less than 6–8 hours and is totally ineffective up to 4 hours. Such a single dose successfully arrests dividing cells up to 16–18 hours after its injection. In these orientation tests on effectiveness of colchicine only single intramuscular injections of 60 bird units of prolactin were used.

When prolactin was thus administered, and its capacity to induce mitoses in crop sacs was studied within the range or time limits of effectiveness of colchicine, such hormone stimulated mitoses in the crop epithelium within 1 to 2 hours after its injection into the pectoral muscles. Promptness of absorption and specific action of prolactin on this tissue is thus indicated. At 6–8 hours after such an injection the number of mitoses sharply diminish although the colchicine is still capable of arresting mitoses; a second injection of prolactin 8–12 hours after the first results in a second and higher degree of cellular proliferation. With subcutaneous injections Leblond and Allen (1937) obtained mitotic stimulation at 36 hours, and in local crop sac tests they observed stimulation as early as 9 hours.

In incubating pigeons (untreated) the earliest positive histological indication of crop sac proliferation, according to Beams and Meyer (1931), appears at about the eighth day. The colchicine technique now shows that an increased mitotic rate is absent until after the laying of the first egg but is 4–6 times the normal (repair) rate after one day of incubation.

*The action of physostigmine and prostigmin on the acetylcholine and nicotine contractures of the rectus abdominis muscle of the frog.* A. M. LANDS (introduced by T. Koppanyi). Department of Physiology, Georgetown University, School of Medicine, Washington, D. C.

It is well established that small amounts of acetylcholine (1 part in 500,000 or less) induce a strong contracture of the isolated rectus abdominis muscle of the frog. Physostigmine sulfate, salicylate or prostigmin (1 part in 500,000 or more) increases the height and/or the duration of the contracture due to acetylcholine. The rectus abdominis muscle preparation of the frog (Rana pipiens) used in our laboratory was found to respond to physostigmine sulfate or salicylate (1 part in 10,000) by a slowly developing contracture, this being associated with a depression of the subsequent response to acetylcholine. The concentration of acetylcholine used to induce a contracture following treatment with physostigmine does not influence the depressing action of physostigmine. The acetylcholine contracture is completely abolished by concentrations of physostigmine slightly greater than those capable of depressing the response to acetylcholine. Prostigmin has the same action as physostigmine except that the concentration necessary for abolition of the response to acetylcholine is approximately 1 part in 2,000.

Following the complete suppression of the contracture by means of physostigmine or prostigmin the rectus abdominis preparation will respond vigorously to a 0.5 per cent solution of potassium chloride. This response is a contracture similar to that induced by acetylcholine. Repeated washing in Ringer's solution reverses the action of physostigmine or prostigmin. Within a few minutes the ability to respond to acetylcholine is regained. It is interesting to note in this connection that the responses return in the reverse order of their disappearance, i.e., depressed response, potentiated response and finally normal response.

Atropine does not antagonize the depression or paralysis produced by treating the rectus abdominis preparation with physostigmine or prostigmin. Effective concentrations of atropine (1 part in 4,000 approximately) prevent or abolish the acetylcholine contracture. No concentration of physostigmine or prostigmin will abolish the atropine paralysis. The response to nicotine following physostigmine or prostigmin is somewhat variable. Although there is no potentiation comparable to that produced for acetylcholine, concentrations of physostigmine and prostigmin which abolish the response to acetylcholine abolish the response to nicotine (1 part in 1,000) in about 60 per cent of the experiments.

*The processes of secretion and synthesis of protein material in the pancreas.* G. O. LANGSTROTH (by invitation), D. R. MCRAE (by invitation) and S. A. KOMAROV. Departments of Physics and Physiology, McGill University, Montreal, Canada.

The concentrations of Na, K, Ca, Cl, $HCO_3$, and protein and nonprotein nitrogen were determined in series of samples of pancreatic secretion obtained from dogs in response to (a) a constant rate of administration of secretin, (b) a varied rate of administration of secretin, or (c) interrupted administration of secretin (rest period, 2 hours). The use of quantitative spectroscopic methods of analysis in the determination of the metals, and of absorption spectrum measurements in the study of the

protein composition of the secretion, were important features of the technique.

The most striking point in the research is concerned with theoretical considerations of the processes of synthesis and secretion of protein material by the gland. A mathematical treatment of these processes is based in part on the supposition that both synthesis and secretion involve chemical reactions within the glandular cells. In its fundamental aspects the theory is a more general case of that which describes the secretion of protein material by the submaxillary gland in response to chorda tympani stimulation (Langstroth, McRae and Stavraky—in process of publication). It leads to equations which quantitatively describe all the observed characteristic features of the protein output in the samples of the widely varied experiments mentioned above, and permits the calculation of various factors not directly observable in critical experiments. Interpretations based on the theory permit considerable insight into the fundamental nature of certain of the glandular mechanisms, and are rich in suggestions for further experiments.

*Long-lasting effects of activity on ganglionic transmission.* M. G. LARRABEE (by invitation) and D. W. BRONK. Johnson Foundation, University of Pennsylvania, Philadelphia.

An important factor in the processes of cellular excitation is the long persistence of the effects of activity which we have previously observed in the stellate ganglion. In order to analyze these mechanisms we have recorded the action potentials in the postganglionic nerve as an index of the number of ganglion cells responding to a preganglionic volley.

One second after a single submaximal volley 10 to 30 per cent more cells respond to a second similar volley. This period of delayed facilitation begins 0.2 to 0.5 second after the conditioning stimulus and after rising to a maximum at one second slowly declines for more than five seconds. It is therefore much later than the periods of refractoriness, facilitation, and inhibition described by Eccles (J. Physiol. **85:** 207, 1935).

Following rapidly repeated stimulation the delayed facilitation may be as great as 500 per cent and last for some minutes. The magnitude of the effect, the time to its maximum, and its duration increase with the frequency and duration of the conditioning stimulation. The increase in response to a given submaximal testing volley is the same whether the conditioning volleys be greater than or of the same size as the testing volleys.

A short train of antidromic impulses depresses transmission for several seconds after which there is facilitation. If the duration of the antidromic stimulation be increased to five seconds at 20 per second only depression follows and this may last for several minutes. The effect of antidromic stimulation thus contrasts with that of dromic conduction and suggests that the long-lasting facilitation is the result of preganglionic activity whereas postganglionic activity produces only depression. This depression is presumably masked by facilitation when the conditioning stimuli are preganglionic.

It is suggested that these prolonged effects may be due to the persistence of chemical substances liberated at the synapses or to an oxygen debt developed during activity. In agreement with this hypothesis is the fact that the phenomena are more pronounced in a non-circulated ganglion.

*Distention and inflammation as stimulating agents for uterine motility.* MAURICE LAUFER (by invitation) and SAMUEL R. M. REYNOLDS. Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y.

Chronic distention of the uterus with paraffin pellets of suitable sizes induces in adult, ovariectomized rabbits a series of histological changes which includes hypertrophy and hyperplasia of the uterine muscle. The effect is attributable to an optimal degree of tension being imposed upon the tissues, since too little or too much tension fails to elicit the distention-growth response, and since progestin diminishes uterine tone and renders the uterus less sensitive to distention.

Experiments were carried out to test the activity and reactivity of the uterus in adult rabbits three weeks after ovariectomy and four days after distention of the uterine lumen by saline. One horn was untouched, for control purposes. Motility records were made simultaneously from each horn in the unanesthetized animal. No motility was found in either horn so long as the uterus was free from inflammatory processes, nor was it reactive to oxytocin or pilocarpine. The uterine enlargement of the distended horn was found to be from 75.6 per cent to 108.3 per cent. In those cases in which inflammatory processes were found in the vascular zone of the myometrium, the uteri were highly but irregularly active. Pilocarpine was without effect. When motility is induced by oestrin, it is rhythmic and the myometrium responds to pilocarpine. Uterine growth resulting from simple distention alone, however, is unassociated with any increase in spontaneous myometrial activity or responsiveness to highly oxytocic agents.

*The modification of spontaneous cortical activity by sensory stimuli.* F. H. LEWY and G. D. GAMMON (by invitation). Neurological Institute, University of Pennsylvania, Philadelphia.

The cortex of the cat under nembutal anesthesia exhibits bursts of rhythmic activity at irregular intervals. This study aimed to determine if this activity were dependent on the normal sensory inflow. Since we were interested in the response of the cortex as a whole rather than that of the primary sensory areas, leads were taken from the suprasylvian gyrus behind the sensory area. The waxing and waning rhythm could be abolished by section of the brain stem at the level of the colliculi, by destruction of the optic thalamus or by interruption of thalamo-cortical fibers in the internal capsule. Progressive diminution of the bursts occurred when successive cuts were made through the spinal cord, medulla and pons. On the other hand, removal of the cerebellum, opposite hemisphere, both occipital or both frontal lobes did not abolish the rhythm. These observations thus suggest that the spontaneous rhythm is dependent in some way upon the integrity of the normal sensory pathways. This dependence is more marked in nembutal anesthesia than in the unanesthetized cortex which is in conformation of Bremer's experiments. Even there, however, the pattern is profoundly altered.

To determine how sensory stimulation altered the spontaneous cortical activity, sensory nerves, the thalamus, and thalamo-cortical fibers were stimulated. By this means burst activity could be driven by repetetive single shocks, the bursts usually escaping from the drive after some minutes. At the end of stimulation long inhibition usually followed. If

the cortex showed no activity, the stimuli aroused a waxing and waning rhythm lasting some minutes; if activity was minimal, amplitude and frequency could be increased.

These two lines of evidence support the conception that the spontaneous activity of the cortex is modified by impulses which constantly go into the brain from the body. This effect is apparent not only in the primary sensory areas but also in other parts and possibly in the cortex as a whole.

*Chemical control of potentials of the isolated frog brain.* B. LIBET (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago, Ill.

The olfactory bulb of the isolated frog brain maintains a remarkably regular potential rhythm and affords an excellent preparation for study of the metabolism underlying automaticity of and synchronization among neurones. Potentials were led by fluid or metal electrodes into a push-pull amplifier and recorded with a crystograph. The influence of desired agents was determined by bathing the preparation in appropriate solutions.

The $Q_{10}$ of both frequency and amplitude averaged 2.3. Raising temperature increased regularity (improved synchronization).

$Ca^{++}$ or $Mg^{++}$ caused reversible slowing of the 6/sec. waves, definite at 0.001M (added to Ringer), and progressive with concentration to 30 per cent slowing at 0.007M. Stronger $Ca^{++}$ (0.018M) caused a discontinuous change to waves at 1/sec., at the original amplitude, with small irregular 10–20/sec. waves superimposed. $K^{+}$ (0.002M in Ringer) reversibly speeded up the 6/sec. waves by 20 per cent, especially if the normal rhythm was somewhat irregular. Larger concentrations (0.01M) caused a discontinuous change to feeble and irregular rapid waves (15–35/sec.). $K^{+}$ and $Ca^{++}$ partially balanced each other's effects. Sulphate (0.04M), a $Ca^{++}$ remover, acted like excess $K^{++}$ to markedly decrease amplitude, e.g., from 35 $\mu$v to 5 $\mu$v and disrupted the rhythm into irregular waves (30–50/sec.). Other ions added to Ringer (0.01M $Na^{+}$; 0.06M $HPO_4^{=}$, and 0.013M $H_2PO_4^{-}$) or pH changes between 5.9 and 7.8 had no effect. Doubling osmotic pressure, by dextrose addition, in some cases increased amplitude (45 $\mu$v to 120 $\mu$v), regularity, and wave length (0.2 sec. to 0.25). Dextrose substituted for half the salt in Ringer had no effect.

Nicotine (0.5 per cent) gave very regular large slow waves, although it paralyzes central synaptic transmission; so neurones can beat and synchronize without neural stimulation. Iodoacetate (0.004 M) gave fast waves at 20–25/sec. with complex large slow waves accurately repeated at regular intervals (e.g., every 12 sec.). Cyanide, up to 0.1 M, gave a rapid regular rhythm at 20/sec. persisting for hours. These results suggest that the character as well as amount of a neurone's metabolism influences its rhythm.

After the isolated olfactory bulbs had ceased activity, faradic stimulation of the olfactory nerves resulted in reappearance of the slow rhythm; evidence of some dependence of excitatory state on incoming impulses.

*Effect of Na, Ca and K on the isolated perfused coronary vessels.*[1] E. LINDNER (by invitation) and L. N. KATZ. Cardiovascular Dept., Michael Reese Hospital, Chicago, Ill.

[1] Aided by the A. D. Nast Fund for Cardiac Research and Grants no. 237 and no. 283 from the Therapeutic Committee of the A.M.A.

The studies were made on the isolated coronary system of the dog's heart after ventricular fibrillation had been induced. The coronary vessels were perfused via the aorta at constant pressure and temperature with defibrinated, heparinized dog's blood. Coronary flow was measured by the drainage from the pulmonary artery, this being taken as an index of coronary calibre. At the beginning of all experiments, as part of the routine, 10 cc. of 10 per cent calcium gluconate was added to the perfusate.

Various concentrations of calcium, potassium or sodium chloride were added to the perfusing blood. Both the sodium and calcium salts caused coronary dilatation in all concentrations employed (viz., 1:3200 to 1:250 for $Na^+$ and 1:10,000 to 1:270 for $Ca^{++}$). In smaller concentrations $K^+$ also caused coronary dilatation (i.e., 1:3000 to 1:670), but in larger concentrations (i.e., 1:930 to 1:360), coronary constriction, sometimes to so extreme a degree, as to result in cessation of coronary flow. Apparently the salt balance present in the blood perfusate at the onset of the experiment was optimal for the greatest degree of coronary vasomotor tone other than the extreme constriction with potassium excess.

In the course of this study, the addition of these salts caused changes in the character of ventricular fibrillation and, at times, ventricular standstill. No consistent correlation could be established between the character of ventricular activity and coronary flow. This would indicate that the coronary flow in our preparation was not affected by the type of ventricular fibrillation or its absence.

*The excitability of inferior mesenteric ganglion cells following a preganglionic volley.* D. P. C. Lloyd (introduced by V. E. Henderson). The Laboratory of Physiology, Oxford, and the Department of Medical Research, University of Toronto, Canada.

Recruitment of $S_2$ cells in the inferior mesenteric ganglia cannot be demonstrated either by the two volley technique or by repetitive stimulations. However, the early part of the inhibitory curve reveals an increasing phase which cannot be attributed to the inhibitory process itself. This effect has been called submerged facilitation, but since it is found in the absence of true facilitation and is always associated with the discharge of an impulse, which is not true of facilitation, it must be regarded as a supernormal phase. The existence of a "subliminal excitatory state" is inferred to explain the prolonged summation effects of the subliminal fringe. Thus the C.E.S. of ganglion cells consists of two states, supernormality and subliminal excitatory state.

Inhibition is an inconstant finding in the I.M.G. In some experiments it cannot be demonstrated. In others it may reduce the ganglion cell response by 25 per cent at the optimal interval. Relative inhibition remains constant as the strength of the testing preganglionic volley is decreased. Thus the impulses in single preganglionic fibres must be, for the most part, individually supermaximal for the ganglion cells as supermaximality by virtue of convergence would be revealed by increased inhibition with weaker preganglionic volleys. In spite of the variability of inhibition the P wave is a constant finding, being equally well developed in those experiments which do not show inhibition. It must therefore be considered as the potential sign of a subnormal phase, rather than of inhibition itself.

Thus the fundamental and constant reactions, i.e., supernormality and subnormality, of ganglion cells may be compared with those of nerve fibres, while the secondary phenomena, facilitation, inhibition or inhibition-resistance, depend upon the complexity of relationship between the cells and their preganglionic fibres.

*Seasonal and menstrual variations in basal oxygen utilization under conditions of controlled temperature, humidity and diet.* JULIA E. LOCKWOOD (by invitation) and FRED R. GRIFFITH, JR. University of Buffalo, N. Y.

These observations were obtained under carefully controlled environmental conditions and a large number of consecutive readings made in an effort to obtain values representing only inherent physiological states. A balanced adequate weekly diet was determined for each subject (4 medical students, 2 women and 2 men respectively) for consumption throughout the experimental period of 14 months. The subjects slept in air-conditioned rooms where humidity and temperature were held constant throughout the year. The activity was controlled only to the extent that the programs of the students permitted. The basal metabolism was taken on each subject six times weekly—the same time each morning when the subject had just awakened.

Under these conditions, the average oxygen utilization expressed in cc. of $O_2$/min., showed a definite seasonal and menstrual variation. The total seasonal variation of approximately 3.5 per cent has the low part of the curve in the fall and the high in the spring. The menstrual variation of about 6 per cent shows its low value at the 8th day of the period and the highest points just before the onset of menstruation.

*The effect of adrenalectomy and adrenal cortical hormones upon pancreatic diabetes in the rat.*[1] C. N. H. LONG, E. G. FRY (by invitation) and K. W. THOMPSON[2] (by invitation). Laboratory of Physiological Chemistry, Yale University School of Medicine, New Haven, Conn.

We have performed partial pancreatectomy on over one hundred rats at an age of 25–30 days by the method of Pincus and Shapiro. The mortality is small, and on reaching an age of about 100 days it was found that some 30 per cent of them exhibited glycosuria when fed (1–10 grams daily). In a given rat, on a constant diet, the daily glycosuria was remarkably constant.

The remaining rats that did not exhibit glycosuria were injected with a crude saline extract of anterior pituitary (70 mgm. gland per 100 gram rat). A large number of these promptly developed either a temporary or permanent glycosuria. This type of animal is very useful for testing the glycosuric action of various glandular extracts.

Some of the animals exhibiting spontaneous glycosuria were adrenalectomized and maintained on saline (50–100 cc. 1 per cent NaCl daily). Although the animals remained in good health, as shown by their survival and excellent appetite, the glycosuria either disappeared or was greatly reduced.

[1] This work was materially assisted by grants from the Fluid Research Fund, Yale University School of Medicine and the Endocrine Committee, National Research Council.

[2] Alexander Brown Coxe Fellow, Yale University.

The glycosuria could be made to reappear (a) by grafting the adrenals of day old rats (b) by the peroral daily ingestion of 5–10 cc. of Upjohn's cortical extract[3] (c) by the peroral daily ingestion of 3 mgm. of the crystalline compounds A or B isolated from the adrenal cortex and kindly supplied to us by Dr. E. C. Kendall.

These experiments have convinced us that the effects of adrenalectomy on pancreatic diabetes are due to the loss of the same hormone as is responsible for life maintenance and for a normal electrolyte metabolism. The ingestion of sodium chloride, although capable of substituting for the cortical hormone in the last two instances, is not capable of restoring glycosuria.

The effect of this hormone on carbohydrate metabolism is further shown by the increased glycosuria that occurs in partially depancreatised (but otherwise normal) rats when large quantities of either the extract or crystalline compound is given by mouth.

*Subliminal stimulation of medullated frog nerve.* R. LORENTE DE NÓ. Laboratories of The Rockefeller Institute for Medical Research, New York City.

The subliminal cathodal curve is not a mirror image of the anodal one. For a given interelectrode distance the duration of cathodal summation increases with the shock strength, while the duration of anodal depression is independent of the strength. If the interelectrode distances are short enough, anodal depression may last for a shorter time than the applied shock. For a given shock strength the duration of both cathodal summation and anodal depression increases with the interelectrode distance. Rhythmic subliminal stimulation causes at the cathode a progressive decrease of the duration of the period of local summation after the successive shocks, and an increase of the depth of the postcathodal depression. Under given conditions the postcathodal depression may be followed by a second period of summation. The cathodal and anodal effects are decrementally propagated away from the electrodes, summation of two subliminal shocks being possible with cathodes 12–15 mm. apart.

The time course of the cathodal and anodal effects varies with the distance from the electrodes; the maximum appears the later, the greater the distance. Thus at the time at which the postcathodal depression is starting under the cathode, several mm. ahead of the cathode, the rise in excitability may be passing through its peak. Experimental time-space-excitability surfaces will be presented.

When stimulation is effected with two circuits having a common cathode, i.e., one cathode between two anodes, a subliminal shock through one circuit increases the response to a submaximal shock through the other circuit. On the other hand, in the symmetrical case of one anode between two cathodes the subliminal shock decreases, or even suppresses the response to the submaximal shock. The strength and time course of these effects are dependent on the strength of the subliminal shocks and the distances between the electrodes of the two circuits.

*The antiketogenic and glycogenic activity of succinic acid.* EATON M. MACKAY and RICHARD H. BARNES (by invitation). The Scripps Metabolic Clinic, La Jolla, Calif.

[3] We are indebted to Dr. G. Cartland of the Upjohn Company for this extract.

Contrary to the observations of Koranyi and Szent-Györgyi (Deutsch. med. Wchnschr. **63:** 1029, 1937) succinic acid was found to be without effect upon the ketosis of partially controlled diabetic patients in whom the ketonuria quickly disappeared with a small increase in the dosage of insulin. When given to a normal fasting person or one receiving a ketogenic diet this substance reduced or abolished the ketosis as does glucose. Administered to phlorhizinized dogs succinic acid was entirely converted to glucose. Under similar conditions the ketonuria of fasting rats fed $\beta$-hydroxybutyric acid or of rats fasting after a period on a high fat diet low in protein was reduced in practically the same degree by succinic acid or an equivalent quantity of glucose. The glycogenic activity of succinic acid and glucose is the same as measured by the deposition of hepatic glycogen in rats after the oral administration of comparable amounts of these substances.

*The influence of adrenal cortex extract upon compensatory hypertrophy of the adrenal cortex.* Lois L. MacKay (by invitation) and Eaton M. MacKay. The Scripps Metabolic Clinic, La Jolla, Calif. (Read by title.)

The compensatory hypertrophy of the adrenal cortex which occurs after the removal of one gland is reduced or entirely prevented by the administration of a potent extract of the adrenal cortex. In a typical experiment the average body weights and right adrenal weights for four groups of seven female rats ten days after operation were as follows: Control group 99 grams and 18.3 mgm., adrenal cortex extract treated control group 93 grams and 15.1 mgm., operated group with left adrenal removed 99 grams and 31.1 mgm., adrenal cortex extract treated operated group 98 grams and 21.0 mgm. Histologic evidence supported the weight data. The extract treated groups were given 0.5 cc. twice daily of a cortical extract (Wilson Laboratories) of known potency. It is concluded that the stimulus to compensatory hypertrophy of the adrenal cortex is brought about due to an insufficient concentration of the cortical hormone circulating in the organism. It is probable that the hypertrophy is controlled by the adrenotropic principle of the anterior pituitary for it has been shown (Ingle and Kendall, Science **86:** 245, 1937) that administration of large amounts of cortical extract cause atrophy of the cortical tissue in normal rats but that this atrophy may be prevented by the simultaneous administration of the anterior pituitary adrenotropic fraction.

*A note on glycogen purification.* J. J. McBride (introduced by Ernest L. Scott). Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

Following alcoholic precipitation of purified glycogen specimens, it is customary to dry down to constant weight in vacuo. It appears that when calcium chloride is used as the desicant at room temperature there may remain adsorbed on the glycogen considerable alcohol and possibly traces of impurities from the latter. This contamination results in fictitious glucose yields when the reducing sugar is determined after hydrolyzing these specimens.

The following procedure can serve as a criterion of the completeness of drying: A weighed amount of glycogen is hydrolyzed by acid and the glucose is determined by the method of Shaffer Hartmann. Another portion of the same specimen of glycogen of equal weight is run through

the modified Pflüger method of Blatherwick et al. (J. Biol. Chem. iii, 1935) and following hydrolysis, the glucose is determined as before.

When this procedure is applied to a specimen thoroughly dried at 0.1mm. over $P_2O_5$, the two glucose values are identical. An imperfectly dried specimen, however, may show an apparent glucose yield, following direct hydrolysis, from 3 to 9 per cent higher than the value obtained in the second step.

If a freshly dialyzed solution of glycogen is treated in the same way, the glucose yields from the two steps are identical, showing that the alcoholic precipitation is responsible for the discrepancy.

The glycogen specimens used in the work were prepared from cat and rabbit livers and purified by the method of Somogyi (J. Biol. Chem. 104, 1934).

*Effect of local application of progesterone on the rabbit uterus.* D. A. McGinty, L. P. Anderson (by invitation) and N. B. McCullough (by invitation). Research Laboratories, Parke, Davis and Company, Detroit, Mich.

Characteristic effects of theelin and testosterone, which may be elicited by application locally to tissues which respond to these hormones, occur ordinarily with smaller doses than those which are required to produce the same reaction when administered parenterally. Similar results have been obtained with crystalline progesterone.

Immature rabbits of 1 kgm. average weight, after preliminary treatment with theelin—150 I.U. over 6 days—were anesthetized with ether, the abdomen opened and the uterus exposed. Ligatures were tied at each end of 3–4 cm. segments of each horn, care being taken to avoid disturbing the blood supply to the tied-off loop.

Crystalline progesterone in 0.1 cc. peanut oil or lanolin solution was introduced into the cavity of the isolated segment. The same volume of oil or lanolin alone was placed in the corresponding segment on the opposite horn. The abdomen was closed.

The rabbits were killed at 72 hours. Several portions of each horn in the uninjected as well as injected regions were removed for histological examination.

Doses of 0.5 to 5 gamma progesterone in 3–4 cm. of uterus (22 animals) caused characteristic epithelial changes equivalent to those brought about by 500 gamma of hormone given intramuscularly (hind leg) in divided doses for 6 days. As little as 0.12 gamma (4 animals) produced definite progestational growth. Doses of 5–20 gamma in all but 2 out of 20 animals were equivalent in their effect on the experimental segment to the intramuscular injection of 0.5–1.0 mgm. of hormone. In no case did any portion of uterus in the control oil or lanolin injected segments or in uninjected regions on either horn show any trace of progestational proliferation. Bilateral ovariectomy (3 animals) failed to alter the response.

*Junctional facilitation and inhibition following a single motor nerve volley.* C. A. Maaske (by invitation), T. E. Boyd and John J. Brosnan (by invitation). Department of Physiology and Pharmacology, Loyola University School of Medicine, Chicago, Ill.

1. Facilitation. In dogs under nembutal anesthesia, response of the tibialis anticus muscle to single efferent volleys was suppressed by (a) curare, (b) magnesium salts or (c) excess of acetylcholine after eserine.

The duration of the facilitation left by the first of two volleys is approximately the same in (a) and (b), and in neither case is it prolonged following eserine administration with recurarization to the original state. This confirms recent findings of Bremer and Kleyntjens (Arch. Int. de Physiol., **45**: 382, 1937) on curarized frogs. In our experiments absence of lengthening of the facilitation period was not due to rapid disappearance of the effects of eserine. This was proven by the prolonged decurarizing action of ACh in doses which were ineffective before eserine.

In (c) above, facilitation apparently is absent. When stimulation of the nerve at 2 per second failed to evoke responses, two stimuli 5 ms. apart were equally ineffective.

2. Inhibition. After doses of curare smaller than those used in (1), the response of the muscle to the second of two nerve volleys is depressed or fails entirely if the interval between is somewhat longer than the facilitation period. This after-depression lasts at least 10 seconds (Rosenblueth and Morison) so that with continued stimulation of the nerve sustained Wedensky inhibition, partial or complete, is produced. Under Mg the after-depression is relatively slight, lasts not more than 2 seconds, and disappears entirely after a brief series of volleys. Wedensky inhibition therefore fails to appear with any frequency of stimulation up to 120 per second.

*Influence of cobra venom on action of some convulsant drugs.* DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The author has shown experimentally that the analgesia produced by cobra venom injections is due to their sedative effect on the brain. Further research also revealed that in suitable doses the venom antagonizes to some extent the epileptiform convulsions produced by camphor. An extensive investigation has been carried on with a series of drugs and chemicals which in suitable doses produce convulsions of different physiological types. The effects of camphor, borneol, cocaine, atropine, coramine, metrazol, picrotoxin, phenol, absinthe, and caffeine *on mice* were thus investigated. The action of some of these was found to be antagonized or neutralized by subsequent injections of cobra venom; that of others was unaffected. In still other cases, however, synergistic phenomena resulted from administration of cobra venom after the other drug. No synergism between cobra venom, on the one hand, and strychnine or phenol, on the other, was noted. Cocaine and cobra venom were usually synergistic, effecting greater toxicity. Absinthe and picrotoxin convulsions were definitely counteracted or much weakened by cobra venom injections. The data so far obtained support the view that cobra venom in suitable doses depresses the higher regions and synapses of the central nervous system, acting on both the cerebrum and medulla. These findings throw some light on the mechanism of the favorable symptomatic action exerted by the drug on the Parkinsonian syndrome, first pointed out by the writer and recently confirmed and extended by R. Finley Gayle (Southern Med. J., February, 1938).

*Influence of barometric pressure on narcotic action of tri-brom ethanol.* DAVID I. MACHT. Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

When applied to the intact skin of mice and rats, both tri-brom ethanol and tri-ethyl ethanol are rapidly absorbed, producing narcosis. Experiments with tri-brom ethanol thus administered revealed that the minimal narcotic dose of the drug varies with the barometric pressure. The usual minimal quantity of a 10 per cent solution in 95 per cent ethanol required to produce anesthesia by application to the skin was 0.80 cc. for a standard mouse weighing twenty-four grams. This dosage, obtained in Baltimore at sea level, was not the same as that obtained in a series of experiments performed at the Maryland State Sanatorium in the Blue Ridge Mountains. It was found that at the lower barometric pressure, the minimal lethal dose absorbed through the skin was 0.90 to 1.00 cc. or more. The influence on dosage of various meteorological factors, especially sudden fall in atmospheric pressure at the time of a storm, is under investigation. The findings already obtained indicate that when there is a profound fall in the barometer a larger dose of the drug is required to produce narcosis, a phenomenon similar to that observed by Nedzel in his studies on morphine.

*Some new physiological and toxicological effects of Roentgen rays.* DAVID I. MACHT and CURTIS F. BURNAM (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., and the Howard A. Kelly Hospital, Baltimore, Md.

Zoöpharmacological and phytopharmacological studies revealed new biological effects of Roentgen rays. In animal experiments, normal blood was obtained from dogs, cats, rabbits, guinea pigs, rats, mice, pigeons, frogs and fish, and was compared by methods developed by the author (Protoplasma xxvii, 1, 1937; Arch. Dermat. and Syph. **36:** 1022, 1937) with blood taken from the same animals after their exposure to various doses of x-rays. The most important finding was the discovery in the blood of irradiated animals of a toxic substance not present in that of the controls. This phenomenon, which is best demonstrated not immediately but twenty-four hours after irradiation, persists for several days. In rat experiments, the amount of toxic substance thus generated varies with the part of the animal to which x-rays are applied, the most toxic blood being obtained after irradiation of the head, the next most toxic after irradiation of the abdomen and the least toxic after irradiation of the thorax. Smooth muscle from various organs of irradiated animals gives less pharmacological response to epinephrine and other drugs than the same tissue from identical organs of untreated controls. Studies on oxidation and reduction in freshly excised brain tissue by a modified Thunberg method (J. Am. Chem. Soc. **58:** 729, 1936) revealed transient impairment of enzymatic activity in brains from irradiated animals as compared with those of controls. Clinical studies on blood of patients undergoing x-ray therapy for malignant and non-malignant disease are in progress. Examination has also been made of some drugs which may counteract the toxicity of x-rays.

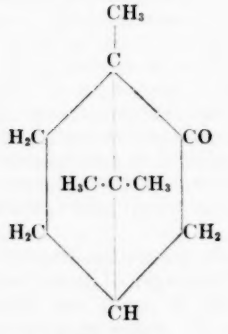
Plant-physiological experiments were made on germination of various seeds, and on root growth of *Lupinus albus* seedlings. Growth of *Lupinus albus* seedlings exposed to from 300 to 500 R units was accelerated. Larger doses inhibited root growth. Suitable doses of Roentgen rays increased percentage of germination of seeds of wheat, oats, tomato and *Lupinus albus*, and also accelerated growth of the embryo plants. Larger doses produced inhibitory or toxic effects.

*Toxicological studies on congo red.* DAVID I. MACHT and MARY LOU GRUMBEIN (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

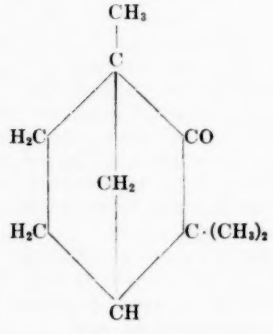
This drug, employed as a physiological reagent and in clinical tests for estimation of the functional state of the reticulo-endothelial system and blood volume and as a test for amyloid disease, etc., is being introduced as a chemotherapeutic agent in certain infections. Further information concerning various samples on the market is therefore desirable. The toxicity of the dye is much greater than has been supposed and various samples obtained on the market differ in potency. When the different specimens of the dye were tested, it was found that the fatal dose after single intraperitoneal injections in mice weighing twenty-four grams varied from 5 to 10 mgm. Daily subcutaneous or intraperitoneal injection of even smaller doses (2 to 3 mgm.) also produced death after several days. Five cubic centimeters of one per cent solutions, injected intravenously in cats under ether, depressed the respiration and circulation. Continuous intravenous injection of from 10 to 20 cc. of a one per cent solution per kilo weight of cats under ether anesthesia produced death, and lethal dosage varied with the different specimens of the dye examined. The depression of circulation and respiration may be attributed to the intrinsic toxicity of the Congo red molecule and also probably to the effects of the dye on blood coagulation. The authors have confirmed the findings of Taliaferro and Haag (Am. J. Med. Sci. **193:** 626, 1937), who discovered that Congo red in certain concentrations promotes coagulation of the blood while in larger doses it inhibits it. This prompted an inquiry as to the antidotal properties of heparin and it was found that previous injection of heparin reduced the toxicity of the dye for cats at least 50 per cent.

*Pharmacodynamic comparison of camphor, fenchone, borneol and fenchyl alcohol.* DAVID I. MACHT and E. EMMET REID (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

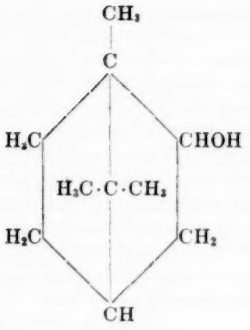
These four compounds are chemically closely related, as the following structural formulae indicate.



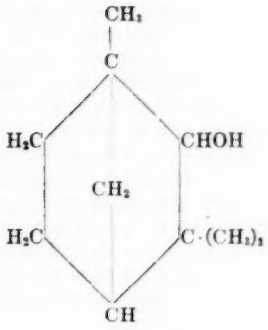

*Camphor*




*Fenchone*




*Borneol*




*Fenchyl Alcohol*

Physiological action of the respective drugs in alcoholic and oil solutions was studied on tadpoles, goldfish, frogs, mice, cats and seedlings of *Lupinus albus*. In experiments on tadpoles placed in solutions of 1:2,000 with 0.2 per cent alcohol and on goldfish in concentrations of 1:1,000 to 1:2,000 fenchone was found to paralyze the respiration more quickly than camphor. Of the two ketones, fenchone was the more poisonous and of the two alcohols borneol was the more toxic. In experiments on mice injected with oil solutions it was found that camphor was the most *convulsant*, fenchone being next in order of such potency, then borneol and fenchyl alcohol. In studies with 0.2 per cent alcoholic solutions of the drugs, fenchyl alcohol and fenchone exerted a mild local anesthesia and camphor and borneol scarcely any. When tested on *Lupinus albus* seedlings, placed in one per cent alcoholic solutions, borneol proved to be the most toxic for growth, next in order being camphor, fenchyl alcohol and fenchone. Conversely, in cats under ether anesthesia fenchone and fenchyl alcohol, injected intravenously, were more depressant than camphor and borneol for the circulation and respiration.

*Physiological properties of some 2.2.2 trialkyl ethanols.* David I. Macht and R. V. Rice (by invitation). Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

Derivatives of ethyl alcohol, analogous to tri-brom ethanol ($CBr_3CH_2OH$), were made by substituting methyl and ethyl radicles for bromine. Thus were obtained tri-methyl ethanol, tri-ethyl ethanol, di-methyl-mono-ethyl ethanol and di-ethyl-mono-methyl ethanol. The physiological action of these compounds was studied on seedlings, on goldfish and tadpoles, on mice, rats, guinea pigs and cats. When one per cent solutions of the respective compounds in oil were injected, it was found that the most narcotic was tri-brom ethanol. Tri-ethyl ethanol was next in narcotic potency and the tri-methyl compound was least narcotic. The di-ethyl-mono-methyl and di-methyl-mono-ethyl ethanols were the most toxic for goldfish and tadpoles placed in solutions of 1:1,000. When tested on seedlings of *Lupinus albus*, the most toxic of the compounds was found to be the tri-brom derivative. Toxicity of the other compounds

varied in proportion to the number of ethyl groups they possessed. Injection of 50 mg. of the respective compounds in 4.50 per cent alcohol solution depressed the circulation of cats under ether. The most toxic was the dimethyl-mono-ethyl ethanol, which quickly paralyzed the respiration and arrested the heart. Studies on muscle oxydases indicated that the tribrom compound was the most inhibitory, the next in order of toxic potency being the tri-ethyl ethanol. In psychological experiments, rats trained in a circular maze, unlike mice, exhibited greater depression after injection of the tri-ethyl compound than after that of the tri-brom ethanol. A most interesting finding was the discovery that 10 per cent solutions in alcohol (95 per cent) of the tri-brom and tri-ethyl ethanols, applied respectively to the intact skin of mice and rats, were rapidly absorbed through the intact skin and produced unconsciousness.

*Effect of histamine, epinephrine and posterior pituitary hormone on behavior of rats in the maze.* DAVID I. MACHT and GIU CHING TING (by invitation). Pharmacological Research Laboratory, Hynson, Westcott & Dunning, Inc., Baltimore, Md. (Read by title.)

The running time and number of errors normally made by albino rats trained in Watson's circular maze were carefully recorded. The behavior of the animals after injection of varying doses of epinephrine, histamine and posterior pituitary (Armour's) was then compared with their normal behavior. Altogether eighteen rats, averaging 150 to 200 grams apiece, were employed. In sufficiently large doses all the drugs depressed the neuromuscular reactions and behavior of the animals. In respect to dosage epinephrine was the most potent while histamine was the least potent. Histamine, 0.1 mgm., sometimes slightly depressed; 0.2 mgm. regularly effected a mild depression while 0.3 to 0.4 mgm. markedly depressed the animals. Posterior pituitary solution was more toxic, as little as 0.05 cc. being sufficient to slightly retard running time. More impairment of the rats' running ability was effected by 0.1 cc. of posterior pituitary, while 0.2 to 0.3 cc. completely disoriented the animals and left them stranded in the maze. Epinephrine was effective in much smaller doses, as little as 0.01 mgm. being mildly depressant; 0.02 mgm. invariably retarded the animals' running time, while 0.03 to 0.04 mgm. exerted a definitely toxic action. Larger doses, such as 0.05 mgm., completely stalled the rats and not infrequently produced paralysis and death.

*A physiological study of the regeneration of the splanchnic nerve in the cat.* J. MAES[1] and F. A. SIMEONE[2] (introduced by W. B. Cannon). Harvard Medical School, Boston, Mass.

The study of completely sympathectomized animals, when pursued over long periods, requires the knowledge of the minimum time necessary for splanchnic nerve regeneration. Fibers from the anterolateral column of the spinal cord are likely to regrow (Hollinshead and Finkelstein, 1937), reestablish connections with the adreno-secretory cells and thus introduce a possible factor of error in the interpretation of certain results.

In 10 cats, therefore, the right and left splanchnics (major and minor) were crushed aseptically in the chest at the point where they branch off from the throacic sympathetic chain. In 8 cats an identical operation

[1] Fellow of the C. R. B. Educational Foundation.
[2] Medical Fellow of the National Research Council.

was performed in the abdomen at the emergence of the nerve from underneath the diaphragm. After 21 to 97 days the animals were urethanized, the two upper abdominal sympathetic ganglia removed on both sides, and the distal end of the splanchnics stimulated in the abdomen or chest with a tetanizing current. The denervated nictitating membrane was used as an indicator of adrenine secretion. Up to 47 days after denervation no evidence of regeneration could be found (11 cases). The first responses were obtained 50 to 63 days after the operations. The height of the response was, as a rule, greater the longer the interval allowed for regeneration. Since the conditions in our experiment were optimal for regeneration (no discontinuity in the nerve), the conclusion may be drawn that up to 6 weeks after sympathectomy no regrowth of fibers to the adrenal is to be feared. Beyond that time, however, the possibility of some regrowth should be borne in mind.

*Nerve cell action potentials of the brain stem.* CONWAY MAGEE (by invitation), JOHN BRICKER (by invitation) and ROBERT GESELL. University of Michigan, Ann Arbor.

Action potentials were recorded from various neuronal structures in the brain stem of the decerebrate dog by means of special needle electrodes and a General Electric multiple oscillograph. The method is capable of registering discrete potentials from pure fiber tracts as well as from regions containing nerve cell bodies (Gesell, Bricker and Magee, Am. J. Physiol. **117:** 423, 1936). By placement of minute lesions and later by histological examination, the site of the potentials can be determined. Potentials have been registered from regions containing dorsal root fibers of spinal nerves, cord arcuates, posterior commissural fibers, the dorsal funiculus of the cord, nucleus gracilis and nucleus cuneatus, the descending root of V and the nucleus descending root of V, inter-olivary fibers, olivocerebellar fibers, the medial leminiscus, the median longitudinal fasciculus, the nucleus fasciculus solitarius, and the brachium conjunctivum; internal arcuate fibers, the medial reticular formation, the lateral reticular nucleus, the medial reticulo-spinal tract and the lateral reticulo-spinal tract; the medial tecto-spinal tract, the dorsal efferent nucleus of X, nucleus ambiguus, root fibers of X, nucleus of VI, nucleus of XII, root fibers of XII, the lateral funiculus of the cord, and ventral horn cells.

The characteristics of the normal discharge and the discharge altered by various chemical and reflex procedures have been analyzed from the point of view of rate, amplitude, discreteness, regularity, recruitment, decruitment, continuity and sporadic appearance. Rhythmic grouping and pattern of discharge associated with the act of breathing are also considered.

*Relation of pH change to the genesis of ischemic work pain.* GEORGE L. MAISON and ARMAND C. FORSTER (introduced by A. B. Hertzman). Departments of Physiology and Surgery, St. Louis University School of Medicine, St. Louis, Mo.

pH changes after ischemic work in human extensor digitorum communis muscle have been studied with the capillary glass electrode in three subjects. The method has been previously described (Maison, Orth and Lemmer, Am. J. Physiol. **121:** 311, Feb. 1938).

After a pH base-line was obtained a pressure of 160 mm. Hg was applied

to the arm while work was being carried out. Work was continued for about 10 seconds after ischemic pain appeared. Simultaneously with the cessation of work, pressure was released, and pH observations resumed. Within 10 seconds the pain disappeared, but after its disappearance maximum acidity was not reached for a period which varied from 13 to 30 seconds in 8 trials. The degree of acidification varied roughly with the work done from 0.11 to 0.37 pH. Return of the pH to its base-line required from 15 to 24 minutes in different trials. A 30 minute rest period was allowed between work trials.

The amount of work done and the degree of acidification resulting was greatest in the first period of work accomplished by each subject. However, the degree of acidification decreased somewhat more than did the amount of work done in subsequent efforts of the same subject, though the pain came on after less work.

The fact that acidification continues after pain disappears, and the fact that smaller acidification can produce the same pain in different trials of the same subject suggest that intercellular acidity cannot be the direct cause of ischemic work pain. Further experiments are in progress to check these preliminary observations.

*Effect of benzedrine and of estrus and pregnancy on the gastric emptying time in dogs.* P. MARCUS (by invitation) and H. NECHELES. Department of Gastro-Intestinal Research, Michael Reese Hospital, and the Department of Physiology, The University of Chicago, Ill.

The gastric emptying time of a meal (consisting of dried whole milk (Klim) barium and water) was determined fluoroscopically. During the course of this work observations were made on the effect of estrus and pregnancy on gastric emptying time.

Benzedrine which is considered by some to release spasm of stomach and intestine was found to produce pyloro- and enterospasm. Estrus definitely prolonged gastric emptying time. Early pregnancy definitely lengthened gastric emptying time, while in the late stage of pregnancy, emptying time returned to control values.

*The effect of anesthesia on the responses of central sensory systems.* WADE H. MARSHALL[1]. Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

The rôle of the anesthetic in modifying cortical potentials correlated with peripheral stimulation has been investigated. In the cat one electrode is placed in the medial lemniscus, one in the thalamic radiations and one on the sensory cortex. The forefoot hairs are stimulated by slight displacements, or the superficial radial nerve is directly stimulated by single shocks. The relative and absolute refractory periods are then determined in terms of potential response at each electrode. Under deep nembutal or dial anesthesia the A. R. P. value for lemniscus is less than 5 msec., below which the determination is indefinite. The potentials of thalamic radiations and cortex cease at a stimulation interval of 50–60 msec. The R. R. P. values are over 200 msec.

Multiple electrodes mounted in trephine holes in the monkeys' skull

[1] Fellow of the National Research Council.

have been used to follow the cortical response to electrical stimulation of a digit, with deep ether, release from ether, or under deep nembutal. Under deep nembutal the A. R. P. is approximately 38 msec. and the R. R. P. 500–700 msec. With deep ether the values are shorter, the A. R. P. approximating 16 msec., the R. R. P. 72 msec. After release of ether, the values are still shorter, the A. R. P. less than 4 msec. and the R. R. P. approximating 17 msec.

The magnitudes of the cortical potentials tend to be smaller under anesthesia, but consistent differences in the latency of the first response have not been observed. These observations suggest that the anesthetic blocks the thalamus by prolonging the refractory period. However, it is possible that failure of the cortex to facilitate the thalamic neurones might be involved. In any case, it is significant that no other characteristic of the cortical response has been found to be modified to a degree comparable with the change in the refractory period.

*Electrocardiographic effects of coumingine hydrochloride, an alkaloid with a digitalis-like action.* S. J. MARTIN and B. COMINOLE (by invitation). Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y. (Read by title.)

Coumingine hydrochloride is the salt of a new crystalline alkaloid extracted from *Erythrophleum coumingo* by Prof. Dalma of Italy. Its potency has been assayed in cats and frogs by Chen and found to be approximately the same as Scillaren A. An attempt in this investigation was made to determine the electrocardiographic effects in cats and dogs. The following preliminary results are reported.

Stock solution of 1:1000 strength was prepared of coumingine hydrochloride crystals dissolved in distilled water. For cats, a 1:100,000 concentration in physiological saline was made and injected intravenously at a continuous rate of about 1 cc. per minute. In dogs, the concentration was 1:50,000 and the rate of injection 2 cc./minute. All injections were made into the right femoral vein under ether anesthesia. Electrocardiograms with leads 1, 2, 3, and 4 were taken before the injections were started and at various intervals after for about 90 minutes. In many instances only lead 2 was taken during the course of injection. These electrocardiograms were compared to those taken on cats receiving one cat unit intravenously of a standardized digitalis preparation.

In preliminary experiments, coumingine hydrochloride was standardized by the Hatcher and Brody method. The cat unit was found to be 0.159 mgm./kgm. confirming the report of Chen. Definite electrocardiographic changes were noted in all cats and dogs employed. In short, these consisted of: initial bradycardia changing later into a marked tachycardia, early increase in height and width of T waves followed by inversion, P–R prolongation, A-V dissociation, ventricular extra-systoles, ventricular tachycardia and finally a ventricular fibrillation. Cardiac disturbances were always seen within 30 minutes when 50 per cent of the calculated cat unit was administered. It is concluded that coumingine hydrochloride, a mineral salt of a crystalline alkaloid, can produce electrocardiographic changes similar to those of digitalis. The duration of the cardiac effects in animals receiving variable sub-lethal doses of coumingine hydrochloride intramuscularly is being studied.

*Syndromes produced in pancreatized cats and dogs as a result of bilateral ligation of the lumbo-adrenal veins.* S. J. MARTIN, J. F. FAZEKAS (by invitation) and H. E. HIMWICH. Department of Physiology and Pharmacology, Albany Medical College, Union University, Albany, N. Y. (Read by title.)

In previous investigations, it was shown that bilateral ligation of the lumbo-adrenal veins ameliorates the course of pancreatic diabetes. In cats, diabetes was not ameliorated in approximately 26 per cent of the animals. The average survival period for 28 cats was 14 days, range 4–31 days. One cat lived 98 days but was not included in this average. In 14 dogs, the average survival period was 13 days, range 4–35 days. In both groups, the animals dying of typical diabetes are included in these averages. No insulin, or cortin, was administered.

Blood studies in 6 cats and 3 dogs in pre-mortal condition revealed that at least 3 different syndromes may develop as a result of pancreatectomy with a bilateral ligation of the lumbo-adrenal veins. (1) Marked hypoglycemia with normal total base noted in 3 cats and 1 dog, (2) normal blood sugar with low total base seen in one cat, (3) hyperglycemia and normal total base, typical of moderate uncomplicated diabetes, in 2 cats and 2 dogs.

An attempt was made to correlate the histological changes of the adrenal and pituitary gland in the 6 cats. The adrenal cortex was normal in the cat showing a diabetic level of blood sugar. Moderate to marked atrophy of the adrenal cortex was found in the remaining five. It is probable that the chemical changes associated with the prolonged survival are related to the degenerative changes of the adrenal cortex. Histological studies of the hypophysis are in progress. These may be significant in view of the findings of Grollman and Firor that ligation of the lumbo-adrenal veins produces pathological changes in the pituitary with secondary hypophyseal dysfunction.

It is concluded that the syndrome produced by a double adrenal vein ligation in pancreatized dogs and cats is a variable one. In 9 animals in which terminal blood studies were made, changes in salt and carbohydrate metabolism occurred independently of each other.

*An experimental study of cardiac pain with reference to the tension factor.* S. J. MARTIN and L. W. GORHAM (by invitation). Departments of Physiology and Pharmacology and Internal Medicine, Albany Medical College, Union University, Albany, N. Y.

The efficacy of a mechanical factor in coronary arteries in the initiation of cardiac pain has been studied in a series of 7 dogs recovering from anesthesia 4–6 hours after surgical preparation according to the Sutton and Lueth procedure. The technique was modified, however, in that 5 black silk ligatures were used in each animal: 3 passing through the adventitia of the anterior descendens branch of the left coronary artery, about 90° apart, and in the same plane, a fourth and more distal ligature underneath this vessel, and a fifth ligature through the myocardium and visceral pericardium. All ligatures were led to the exterior of the thorax through separate flanged glass tubes sewed in place in the parietal pericardium with the proximal ends of the tubes extending freely into the pericardial cavity. Tension upon the ligatures was applied for different periods of time, but was released promptly when the characteristic pain response was noted.

The criteria accepted, signifying the presence of pain in dogs awakening from anaesthesia, were essentially similar to those reported by Sutton and Lueth.

It was found that a characteristic pain response can be elicited when tri-directional tension on the ligatures in one plane (ligatures 1, 2, 3) is applied to a coronary vessel so as to cause no significant change in blood flow. With this procedure the chemical factors caused by impaired blood flow are completely eliminated, as shown by the absence of changes in the electrocardiogram. The minimal threshold of tension on coronary ligatures was 3 grams in the series of dogs used. Tension on the ligature surrounding the coronary vessel (ligature 4) produced pain and electrocardiographic changes within one minute; tension on the myocardial ligature (ligature 5) produced only a sinus tachycardia and no pain. Local application of alcohol was found to block stimuli initiating cardiac pain, whether they be mechanical or chemical in origin. It is concluded that tension on the coronary arteries in dogs may serve as an adequate stimulus in the initiation of cardiac pain.

*Acid—base—water balance.* EDWARD C. MASON and ARTHUR A. HELLBAUM. Department of Physiology, University of Oklahoma Medical School, Oklahoma City.

We have observed the effect of acid and base on 93 subjects and compared the results obtained with those resulting from hyperventilation. Analysis of the results indicate that hydration and dehydration of the body tissues is due to alterations in their hydrophilic properties. The hydration of the tissues is neither an expression of the osmotic changes produced by the salts present, nor is it due directly to the accumulation of acid. The presence of alkali increases the hydration of tissues in the same manner as it increases the hydration of the red blood cell, fibrinogen, caseinogen, stearic acid, urinary casts.

*Do the unicellular organisms produce growth promoting substance?* S. O. MAST and D. M. PACE (by invitation). Zoological Laboratory of The Johns Hopkins University, Baltimore, Md. (Read by title.)

There is much controversy concerning the question as to the production of growth promoting substance by unicellular organisms. The more recent work favors the contention that there is no such substance produced and it indicates that the increase in rate of growth observed, by some investigators, is correlated with changes in the character, the quantity and the availability of the food in the culture fluid.

*Chilomonas paramecium* was used in the following experiments. It grows readily in a sterile solution which contains only relatively simple chemicals in known concentrations. It is therefore well suited for experiments concerning the question under consideration.

Three methods were used: (1) Different numbers of chilomonads were put into the same quantity (0.4 cc.) of culture solution in depressions on pyrex glass slides and counted at different times for 24 hours. (2) One chilomonad was put into each of several depressions containing different quantities of solution (0.006—0.4 cc.) and counted at different times for 24 hours. (3) Different numbers of chilomonads (1–400) were put into the same quantity of solution (0.1 cc.) in different depressions. After 12

hours, 0.006 cc. solution from each was transferred to a clean depression and one chilomonad added, then they were counted every hour for 10 hours.

The results obtained with the three methods are essentially the same. They prove conclusively that, within certain limits, as the number of chilomonads per volume of solution increases, the frequency of fission increases, to a maximum of more than 50 per cent, then decreases to zero.

The results obtained in various modifications of these experiments strongly indicate that *Chilomonas* secretes substance which promotes growth if the concentration is low and inhibits growth and kills the organism if it is high.

*Extracellular fluid from frog muscle.* FRANK W. MAURER (introduced by W. O. Fenn). Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Fluid from the extracellular spaces in frog skeletal muscle can be obtained in amounts varying from 0.5 m to 1.8 cm. The technique consists in piercing the muscles longitudinally with a thin-walled capillary pipette and collecting the fluid lying between the cells by capillary attraction. Histological examination of the muscles shows that the cells are not penetrated by the capillary but change their shape so as to allow free passage of the pipette. The samples of fluid so drawn were analyzed for chloride, protein and pH. The chlorides when compared with the serum chlorides from the same animals, give the following ratio: $\frac{\text{Cl in blood serum}}{\text{Cl in extracellular fluid}} = 0.99 \pm 0.10$. If all the chloride of muscle occurs in similar extracellular fluid this must constitute on the average 19.6 per cent of the muscle weight. The protein content of the fluid is less than that of serum (roughly 1 per cent). The pH of the fluid from normal muscles is $7.4 \pm 0.067$ and from muscles treated with iodoacetic acid is $7.56 \pm 0.103$. Fluid collected however, from freshly cut surfaces has a pH of $6.27 \pm 0.078$ in normal muscles and $7.07 \pm 0.089$ in iodoacetate-treated muscles. At cut surfaces some acid cellular fluid is apparently included.

Acid dyes cannot be used to determine intracellular pH, since they are supposed not to penetrate the cells. To confirm this, frog muscles were placed in solutions of acid dyes, principally phenol red, and the distribution of the dye after equilibration was compared with the distribution of chloride. All the absorbed dye could be accounted for in the chloride space (27.7 per cent) on the assumption that the increase in chloride space (8.1 per cent) during soaking was due to dead cells which concentrated the dye to the same extent (3 times) as does muscle tissue killed by other means. In live muscles the absorbed dye is never sufficient to fill more than 75 per cent of the muscle water, to a concentration equal to that outside.

*The basal metabolic rate of southern Negro females.* MARGARET MAYNARD (by invitation), LOIS C. LEVY (by invitation) and A. G. MULDER. Department of Physiology, College of Medicine, University of Tennessee, Memphis.

The basal metabolic rate of nine southern Negro females aged 20 to 26 was determined in Memphis during the summer of 1937. The group was selected from students at LeMoyne College and was probably superior

financially to the average Negro of this region. Most of them could be classed as light skinned and were probably not of a pure Negro strain.

All of them appeared healthy, their chest and heart were negative on ausculation, their body temperature was normal, their urine showed neither glucose, albumin nor acetone. They reported no disorders of menstruation. Two of them had palpable thyroid glands but are nevertheless included in the series as their basal rates are not greatly different from those of the others.

Four metabolism determinations were made on each individual. The interval between determinations was usually one week. The results of first tests were usually higher than the results of later tests. The last two tests, on an individual, were usually in good agreement. The results when compared with Mayo Foundation Standards are below the normals set by these standards. The grouped results of all tests shows a mean which was 8 per cent below and a median 10 per cent below these standards. If one chooses the lowest results on each individual the mean is 12.3 per cent below the standard.

The average of all pulse counts was 69. The temperature in the room in which the determinations were made varied from 29°C. to 35°C. The diet of the individuals of the group was not determined, none of them appeared to be undernourished.

*A comparison of the blood-cerebrospinal fluid barrier of normal white and negro children as measured by the bromide permeability quotient.* FRED A. METTLER, HENRY G. STELLING (by invitation), MEINHARD ROBINOW (by invitation) and CLAUDE MC.K. BURPEE (by invitation). Child Neurology Research (Friedsam Foundation), Departments of Anatomy and Pediatrics, University of Georgia School of Medicine, Augusta.

Fifty normal children (26 negroes and 24 white) were tested by the Walter bromide test as modified by Katzenelbogen. (The word Negro as herein employed is used in the sense understood by the Southern social system, i.e., any person exhibiting any perceptible admixture of negro blood.) This test is based upon the previous oral administration of a constant (proportional by weight) dosage of sodium bromide over a period of time and the comparison between the amounts of this substance found in the blood and cerebrospinal fluid. It is assumed that the test reflects, to some degree, the functional activity of the barrier between blood substance and nervous tissue. Since the absolute amounts encountered are less significant than the relationship between cerebrospinal fluid bromide and blood bromide it is customary to express the result of the test as a quotient representing the ratio of one to the other. The normal bromide permeability quotient is usually given as varying between 2.7–3.5 (or more frequently 2.9–3.3). As implied above this figure represents the quotient of the division of blood bromide by cerebrospinal fluid bromide.

The above usual accepted "normal" figures represent data insufficiently corrected for age, sex and race and the present research deals with an evaluation of these factors among children. From the data obtained thre conclusions may be drawn, (1) the negro child exhibits a distinctly lower range of P-Q's than the white (2) sex appears to exert no influence upon the permeability quotient (3) the P-Q tends to increase with age, at first at a fairly rapid rate and later almost imperceptibly, if at all.

Especially significant is the percentile distribution of the P-Q's of the

two races studied. Only 9 per cent of white children had a P-Q below 2.9 while 70 per cent of the colored were below this figure. Of the white children 87 per cent had a P-Q of 3.0 or above but only 15 per cent of those who were colored. Seventy-five per cent of white children had a P-Q of 3.1 or above but only 7.5 per cent of the negroes.

*The relationship between the neurocytopathology of pellagra and hematoporphyrin, serum and eosin administration in normal and vitamin A, $B_2$ deficiency protein poor diet.* CECILIA C. METTLER (by invitation), V. P. SYDENSTRICKER (by invitation) and FRED A. METTLER. Departments of Anatomy and Medicine, University of Georgia School of Medicine and the University Hospital, Augusta.

Conflicting reports upon the production of a pellagrinous-like neuropatholocial change in vitamin A deficiency, vitamin $B_2$ deficiency, and also hematoporphyrin administration led the authors to the experimental investigation of the above problem. Sixteen dogs of nearly equal weight were chosen for the experiment. Eight of these were placed upon a vitamin A, $B_2$ free protein-poor diet. Eight were fed upon standard rations. Two of each set received respectively pellagrinous serum, eosin, or hematoporphyrin. Two of each set were kept without treatment as controls. The animals on the normal diet were protected from sunlight, the others exposed. All the animals upon the poor diet lost weight rapidly but none of the experimental animals developed any paralytic manifestations nor evidence of black-tongue.

The course of the animals was as follows. None of the dogs on the balanced diet died except those receiving pellagrinous serum and one eosin animal which developed an abscess. Microscopic examination of the nervous system revealed very slight deviations from the normal picture in the dogs studied. Unfortunately the pellagrinous animals were not in good enough condition to section. All the animals on the deficient diet showed neuropathology histologically. Those receiving pellagrinous serum were the first to succumb, those receiving hematoporphyrin lasted longer than those getting no medication at all.

*The vital capacity of Negro college students.* FRANCES Y. MICHEL and CHARLES W. GILTON (introduced by A. G. Mulder). Department of Biology, LeMoyne College, Memphis, Tenn.

The vital capacity observed in 50 Negro college students, male, non-athletes, is consistently below the so-called normal or standard vital capacity calculated from stem length, standing height, weight, surface area, and chest circumference. Their vital capacity is consistently lower even than that found by Shephard and Myers in men at the University of Minnesota, both highest and lowest figures being below the highest and lowest at Minnesota. The percentage of men having less than 90 per cent of the normal vital capacity, calculated from the above measurements, ranges from 31 to 54 per cent higher than the percentage of Minnesota men in the same group, and the percentage of men in the "under 85 per cent" group from 17 to 43 per cent higher.

The vital capacity of 23 male students, athletes, is likewise consistently lower than that of the Minnesota men, but the magnitude of difference is smaller. In the "under 90 per cent" group there are only 6 to 22 per cent more students, and in the "under 85 per cent" group only 9 to 21 per cent more.

The vital capacity observed in 50 Negro college students, female, compared with that found by Boynton in women at the University of Minnesota shows variation. It is lower in relation to stem length, higher in relation to standing height. Related to stem length, in the "under 90 per cent" group the percentage of Negro women is 18 per cent higher; in the "under 80 per cent" group, 33 per cent higher. Related to standing height, the percentage of Negro women is 16 per cent lower in the "under 90 per cent" group and 22 per cent lower in the "under 80 per cent" group. The difference in vital capacity is less significant when related to surface area and weight. The percentages of Negro women are 23 and 16 per cent respectively higher in the "under 90 per cent" group, but 2 per cent higher and 1 per cent lower respectively in the "under 80 per cent" group.

*Effects of eserine, acetylcholine and atropine on the electroencephalogram.* F. R. MILLER, G. W. STAVRAKY and G. A. WOONTON by (invitation). Department of Physiology, University of Western Ontario, London, Canada.

The electroencephalogram was recorded by the cathode ray oscillograph. The cats were under dial and the rabbits were under dial or local anesthesia.

One per cent. Eserine applied to the cerebral cortex of the cat or rabbit increases promptly the frequency of the waves of the electroencephalogram: many small and medium size waves appear, while the amplitude of the large waves diminishes; there are associated muscular responses in the contralateral limbs; the effects are caused by a local, cortical action of eserine, since the electroencephalogram from the opposite, control cortex remains unaltered.

Two-tenths to 1 per cent. Acetylcholine applied to the eserinized cortex evokes in 2–3 mins. rapid waves of wide amplitude (acetylcholine waves); in the rabbit under local anesthesia there are also movements of mastication, trembling of vibrissae and general body twitchings; under dial the large acetylcholine waves occur but the motor effects are less marked. To evoke the acetylcholine waves the eserine must precede the acetylcholine by a few minutes. The reverse order of application is not effective, nor is acetylcholine alone. However, in the rabbit under local anesthesia acetylcholine on the non-eserinized cortex evokes many small waves and reduces the amplitude of the large waves but does not evoke the largest acetylcholine waves. The effects of acetylcholine alone thus resemble those of eserine alone.

One-tenth per cent. Atropine applied to the eserinized cortex introduces into the electroencephalogram periods of intense hyperactivity, separated by intervals comprising waves of smaller amplitude; the hyperactive waves coincide with periodic contractions in the opposite limbs.

The effects of eserine and acetylcholine are attributable to enhanced, those of atropine to diminished, transmission at synapses. The results may be interpreted in terms of the hypothesis that acetylcholine is the chemical transmitter at synapses. It is also conceivable that there is some other opposed synaptic action of eserine and atropine.

*Relation of ease of body heat loss to general metabolic activity.* C. A. MILLS and CORDELIA OGLE (by invitation). Laboratory for Experimental Medicine, University of Cincinnati, O.

Since most metabolic processes in the body are basically combustive in

nature, it is not surprising to find them dominated by the ease with which body heat may be dissipated. Under conditions of difficult heat loss, growth and rate of development are suppressed, resistance to infection is lowered, basal rate of heat production drops, and available energy falls. As body heat loss is facilitated, all these vital functions rise to higher levels. These changes take place irrespective of dietary control, with all other physical factors except ease of heat loss being held constant.

Even the avenue of heat loss seems unimportant, for just as great metabolic stimulation results from easy heat loss through radiant channels alone as from loss largely through direct conduction to cold air. The severe depression of moist hot air can be completely overcome by allowing free loss of body heat by radiation to cold surfaces, even though the body be still surrounded by the same hot moist atmosphere.

Depression of body metabolism, by making heat loss difficult, forces the individual to live more slowly, on a lower activity level, but he seems definitely inclined to live longer, and to age more slowly. This has also been found true with the metabolic retardation of chronic undernutrition. Depression by heat, however, cannot be overcome by increasing the dietary protein or vitamine intake.

These dominant effects of environmental control over rate of body heat loss have a direct bearing on research results that are based on animal experimentation. The same experimental technique may produce results in winter that differ significantly from those obtained in summer heat, unless care is taken to standardize the environment in which the animals live. Likewise the same technique may lead to different results in Chicago and New Orleans. There seems to exist, therefore, a need for uniformity in the physical environment under which experimental animals are to be kept. Such uniformity should be maintained through the seasons and in all regions if the results of biological research are to be comparable at different times and different places.

*The influence of the anterior pituitary gland on protein metabolism.* ARTHUR MIRSKY and S. SWADESH (by invitation). Department of Metabolism and Endocrinology, Institute for Medical Research, The Jewish Hospital, Cincinnati, O.

In continuation of our studies of the influence of the endocrine system on protein metabolism, we investigated the effects of a neutralized alkaline extract of the anterior pituitary gland, and of ablation of this gland. As in our previous studies, the rate of non-protein-nitrogen accumulation in the blood after bilateral nephrectomy was used as a gauge of the rate of nitrogen metabolism, the normal untreated nephrectomized animal being used as a standard for comparison. The extract was administered subcutaneously twelve hours before nephrectomy was performed.

The administration of the anterior pituitary extract to normal dogs results in a decrease in the rate of protein metabolism, which indicates a "sparing" or storage of protein.

The administration of the extract to normal dogs which are subsequently eviscerated results in an increase in the rate of n.p.n. accumulation as compared to that of the untreated eviscerated dog. This indicates that the extract increases the rate of protein metabolism in the muscles, but that the effect is not apparent in the presence of abdominal viscera.

In view of our previous observations that insulin exerts a pronounced

nitrogen sparing effect, we studied the influence of the pituitary extract on the depancreatized dog. Our preliminary data reveal that in the absence of the pancreas, the anterior pituitary extract increases the rate of nitrogen metabolism instead of decreasing it.

Removal of the anterior pituitary gland results in a decrease in nitrogen catabolism.

Our observations are in accord with those already recorded in the literature, but suggest that the anterior pituitary gland exerts a dual action on protein metabolism. One, a direct stimulation of protein catabolism in the muscles, and two, an indirect stimulation of protein anabolism, through the stimulation of the pancreas. It is suggested that the influence of the pituitary gland on growth may be dependent upon its pancreatropic function.

*A comparison of toxicity with cardiac efficiency effects of several digitalis glucosides.* GORDON K. MOE (by invitation) and M. B. VISSCHER. Department of Physiology, University of Minnesota, Minneapolis.

The recognized lack of correlation between the toxicity assay of different digitalis glucosides and the doses required in therapy indicates the desirability of studies distinguishing between the various effects of the several purified glucosides. An attempt to determine a cardiac efficiency unit of the Digilanids A, B, and C of Digitalis lanata is briefly reported. A series of 55 experiments were carried out on the modified Knowlton-Starling heart lung preparation as described by Peters and Visscher, and in addition, lethal-dose experiments were conducted on 17 intact anesthetized cats.

| DRUG | HATCHER CAT DOSE | C.E.D. HEART | C.I.D. HEART | C.D. C.E.D. | C.I.D. C.E.D. |
|---|---|---|---|---|---|
| | *mgm. per kgm.* | *mgm. per 100 grams* | *mgm. per 100 grams* | | |
| Digilanid A | 0.45 | 1.0 | 0.50 | 0.45 | 0.50 |
| Digilanid B | 0.65 | 2.0 | 0.90 | 0.33 | 0.45 |
| Digilanid C | 0.39 | 0.04 | 0.16 | 9.75 | 4.00 |

C.D., Hatcher cat dose; C.E.D., minimum cardiac efficiency dose; and C.I.D., lowest dose producing rate disturbances in heart lung.

It was found with the heart lung preparation that after a control period of failing efficiency at constant external diastolic volume, adequate doses of these drugs always produced an increase in efficiency, but since the degree of improvement is dependent on many difficultly controllable factors, the absolute minimum dose resulting in a perceptible increase in efficiency is used in the calculations presented in the table. This quantity, the C.E.D. was found to vary markedly between the individual glucosides, although the Hatcher cat dose was not widely different. The C.I.D., or minimum dose producing rhythm or conduction disturbances (extrasystoles or block) fails to correlate with either the cat unit or the cardiac efficiency unit. These wide discrepancies emphasize the importance of studying therapeutic as well as toxic effects in the proper evaluation of the digitalis glucosides.

*A modification of the manometric Van Slyke for use in gas analysis.* J. VICTOR MONKE (by invitation) and DIETRICH C. SMITH. Department

of Physiology, University of Maryland School of Medicine, Baltimore. (Demonstration.)

A modification of the manometric Van Slyke designed to facilitate the introduction of gases into the machine will be demonstrated. The essential feature of the new apparatus is the addition of a gas inlet tube to the upper stop-cock in addition to the usual chamber and waste tube. This eliminates the necessity of using the Hempel burette when a gas analysis is to be made.

Modifications in the shape of the manometric tubing also make it easier to clean the machine. The apparatus is designed for student use.

*Excitability of smooth muscle.* R. S. MORISON (introduced by W. B. Cannon). Harvard Medical School, Boston, Mass.

The activity of the uteri of cats and rabbits was studied in vitro by fixing one horn in the middle and recording the isotonic contractions of both ends. In preparations exhibiting marked spontaneous activity and especially those obtained from animals which had received large doses of esterin, the contractions were well coördinated. Ordinarily one end, usually the ovarian, contracted first and was followed by contraction of the other end, suggesting conduction. Cooling or drying of the muscle at the fixed middle portion resulted in dissociation of the beats. Beats of ectopic origin occurred spontaneously with varying frequency in different preparations, or, especially in the esterinized organs, could be induced by suitable mechanical or electrical stimulation. These were usually, though not always, conducted throughout the muscle.

If adrenine was added to the fluid bathing the leading end of a horn of a non-pregnant cat's uterus, slowing of the rate and decrease of the amplitude occurred, the following side ordinarily showed no change in amplitude but the rate either slowed in coördination with the leading side or took up a new rhythm of its own. Additions of adrenine to the following side produced no change in the leading portion.

In the rabbit adrenine brought about a prolonged contraction of the treated side, usually associated with an increase in the rate of spontaneous activity. The amplitude changes were not conducted to the untreated side, but disturbances of the rate frequently appeared.

An elucidation of the mechanism responsible for the conduction of the spontaneous beats or of the contractions induced by electrical or mechanical stimuli must await further work. It appears, however, that adrenine does not act by setting up all-or-none conducted waves which travel throughout the muscle.

*The use of redissolved dried serum for the replacement of fluid after hemorrhage in dogs.* A. G. MULDER. Department of Physiology, College of Medicine, University of Tennessee, Memphis.

Dried serum was prepared by the method of Flosdorf and Mudd. In this method the water is distilled from the frozen serum in vacuo. This redissolved serum, with its proteins, was injected into bled dogs. Two types of experiments were done, acute experiments and survival experiments.

In the acute experiments the following determinations were made upon dogs anesthetized with Dial: blood pressure, oxygen consumption by spirometer, plasma protein concentration and hemoglobin concentration.

The animals were bled 3½ per cent or 4 per cent of their body weight and, after allowing time for determinations, redissolved serum was injected. In a typical experiment the following values were obtained before bleeding: blood pressure 156 mm. of Hg, oxygen consumption 75 cc. per min., plasma protein N 0.99 gram per cent and hemoglobin taken as 100 per cent. Aftr bleeding 4 per cent of the body weight, 360 cc., these values became: blood pressure 44 mm. of Hg, oxygen use 44 cc. per min., plasma protein N 0.91 grams per cent and hemoglobin 88 per cent. Redissolved serum was injected (300 cc. of fluid, protein N 0.95 gram per cent. The various values redetermined soon thereafter were blood pressure 148 mm. of Hg, oxygen use 77 cc. per min., plasma protein N 0.96 grams per cent and hemoglobin 49 per cent. After 12 hours these values were blood pressure 96 mm. Hg, oxygen use 69 cc. per min., plasma protein N 0.95 gram per cent and hemoglobin 47 per cent. Control animals bled but not reinjected did not long remain in good condition.

In the survival experiments dogs, anesthetized with ether, were bled and reinjected by way of the carotid artery. Their blood pressure returned to normal immediately following the injection but was not followed further. Their hemoglobin and plasma protein values resemble those quoted above but show greater fluctuation. These animals recovered rapidly, they walked soon after the operation. The day following the operation they seemed fairly well and soon completely recovered. Two of these animals died. Several of these animals developed a high fever shortly after the injection one of them dying apparently as a result of the fever.

*Effects of amidopyrine and phenacetin on cutaneous tactile and pain sensitivity.* F. J. MULLIN. Physiology Department of the University of Texas Medical Branch, Galveston.

The effects of amidopyrine and phenacetin on cutaneous tactile and pain sensitivity were determined by the von Frey hair technique on eighteen male subjects. The drugs were administered in five or ten grain doses on an empty stomach. This is the usual dosage generally used for obtaining the analgesic effects of these drugs in cases of headaches, toothaches, rheumatic conditions, muscular pains, colds, etc. However, these doses were without significant effect on the senses of touch or pain from the skin regions of the face and hand in the present tests. In a couple of instances there was a very slight lowering of the threshold to pain, while the larger doses sometimes gave a very slight raising of the pain threshold, but in thirty-seven of the forty-five tests run there were no changes at all in either the tactile or pain cutaneous sensations as measured by this method.

The sensitivity to light touch and to pain was determined for eleven marked spots on the skin of the face and hand before the drug was administered and then at half hourly intervals thereafter for the next two-and-a-half to three hours. Control tests were run with the administration of citrated caffeine or sodium chloride or glucose or nothing at all.

In three instances subjects reported relief from a headache during the running of an experiment on amydopyrine (whether due to the drug or not we can't say), yet in each case there was no change whatever in the skin sensitivity as determined by this technique. Whatever the cause of the analgesic action of these drugs in certain instances, it is presumably not through any change in the mechanism governing cutaneous sensations in response to light touch or a pricking pain.

*Effect on behavior of inhibition of different forms of excitation.* WENDELL MUNCIE (by invitation) and W. HORSLEY GANTT. Phipps Psychiatric Clinic, Johns Hopkins University, Baltimore, Md.

A crucial question confronting the psychobiologist concerns the concept and nature of "inhibition." Possessing no quantitative measure for inhibition comparable to that for excitation, we undertook a study based upon the observed behavior of the total animal.

Sleep has often been noted from inhibition of the food reflex. To test the question, Is sleep a product of all inhibition—blocked activity—or is it specific only for that based upon food excitation, we elaborated inhibitions from opposite types of reactions, viz., to food and to hydrochloric acid. The reaction to food is characterized by the movement of the animal to attain, the reaction to acid to avoid, the object. Anthropomorphically, the former is accompanied by "gratification," the latter by "displeasure." Inhibition of the former leads to a negative state which may be considered anthropomorphically the opposite of the negative state produced by inhibition of the latter. Are the two negative states alike in their effects on the behavior of the animal, or are they unrelated?

A positive conditioned reflex to food was elaborated and afterwards inhibited, leading to sleep, manifested by relaxation, yawning, quiescence, closed eyes. Later, in the same animal a positive conditioned defense reflex to acid was elaborated and also inhibited. Drowsiness, similar to that following the inhibited food signals, followed the inhibitory signal for the defense reflex. Thus, identical states resulted from the blocking of an activity based upon defense and of one based upon food. To this degree we may conclude that "inhibitions" have the common characteristic of causing drowsiness regardless of the underlying excitatory state.

In other animals an attempt to produce inhibition resulted not in sleep and quiescence but in "nervous" disturbances (agitation) lasting for some weeks. The manifestations of blocked activity, "inhibition," are therefore constant and characteristic in a given animal but vary from sleep to agitation in different animals. The individually different reactions point to the existence of functional constitutional differences.

*Observations on the physiology of enterocrinin.* E. S. NASSET, MILTON J. SCHIFFRIN (by invitation) and IRVIN J. BELASCO (by invitation). Department of Vital Economics, University of Rochester, Rochester, N. Y.

Enterocrinin, an intestinal secretory hormone, was prepared according to the method described by one of us (Nasset, Am. J. Physiol. February, 1938). The responses of transplanted segments of jejunum to enterocrinin given intravenously were observed before and after section of the mesenteric pedicle. The same lot of hormone was used for 3 experiments before, and 2 experiments after, section of the pedicle. Doses of 10 mgm. doubled the basal production of succus entericus; 30 mgm. gave a 3 or 4-fold increase.

Acid in the intestine is not the stimulus for the production of enterocrinin, as it is for secretin. HCl given by stomach tube to fistula-bearing dogs or introduced directly into the duodenum in acute experiments does not excite the glands of isolated segments of jejunum to secrete intestinal juice. Parallel observations made on pancreatic fistula dogs have confirmed earlier observations of this type.

The oxygen consumption of isolated dog intestinal mucosa as effected by enterocrinin was studied by the Brodie-Warburg method. The oxygen consumption was increased 7 to 28 per cent depending upon the concentration of hormone. These results are not unexpected since the preparation is a powerful stimulant for the glands of the mucosa.

Enterocrinin as prepared by us does not affect the blood sugar of normal dogs nor does it alter the course of epinephrine hyperglycemia. It does cause a rise of 60 to 100 mgm. per cent in the blood sugar of depancreatized dogs when given in doses of 1 mgm./kgm. intravenously. The maximum effect is seen in 1½ to 2 hours. Three different batches of the hormone have shown this result. It is realized that the hormone preparation is impure and it is impossible to say at present what may be responsible for the change in blood sugar. These findings indicate the absence of "duodenin" (Heller, Wiener Klin. Wchnschr. **44:** 476, 1931) and the probable presence of an "insulin inhibiting agency" (Macallum, Nature **136:** 32, 1935).

*The effect of pituitrine on salivary secretion and on the blood pressure responses to acetylcholine and vagus stimulation.* H. Necheles and F. Neuwelt (by invitation). Department of Gastro-Intestinal Research, Michael Reese Hospital and Department of Physiology, The University of Chicago, Ill.

Dogs anesthetised with pentobarbitalsodium, were injected intravenously with the pressor substance of the posterior lobe of the pituitary gland (pitressin). Doses ranging from 0.005 to 0.1 ccm. inhibit more or less completely the response of the submaxillary gland to stimulation of the chorda tympani. The vasodepressor response to doses of 1–2 gammas of acetylcholine injected intravenously is abolished by the same doses of pitressin as used above. After injection of pitressin doses of 20 to 100 gammas of acetylcholine are entirely ineffective in most dogs. Likewise the cardio-inhibitory and vasodepressor effects from stimulation of the peripheral vagi are inhibited or decreased. Stimulation of the central vagi which, according to Lim and collaborators, produces an output of posterior pituitary secretion, is followed by diminished depressor responses to acetylcholine.

*Probable cause of the St. Louis epidemic of encephalitis of 1933.* Alma J. Neill and Vivla P. Russell (introduced by W. E. Burge). University of Oklahoma, Norman.

Experimentation with white rats, kittens, and monkeys, all of which animals were fed alcoholic extract made from abnormal sweet corn gathered in 1933 in the fields close to St. Louis from which the St. Louis residents were supplied with garden produce, showed that these animals had the same symptoms, the same type of death, and the same physical disorders revealed by post-mortem which the patients had of the St. Louis encephalitic epidemic of 1933.

The strength of the dose, given subcutaneously, affected the onset and severity of the symptoms. Soon the animal's hind legs wabbled when it walked, with complete loss of control resulting; the neck muscles would straighten; there were ocular disturbances; finally the animal would pass into a state of drowsiness or coma.

Although by varying the strength of the dose the character of the symptoms were not changed, the length of the period of coma and the depth of the coma or death could be determined.

In post-mortem the lesions found were in the cortex of the cerebrum, and there seemed to be a tendency toward localization in the medulla oblongata and other parts of the central nervous system. In many cases there was marked inflammation of the kidneys, often with hemorrhage found in the kidney itself.

It is apparent from this investigation that the St. Louis epidemic of encephalitis of 1933 was caused by an alcohol soluble toxin taken into the digestive tract through food, especially, some of the economic plants belonging to the Mayadaceae.

*Changes in reflexes during prolonged stimulation of the afferent nerve.* CLARENCE NELSON (by invitation), DAVID MCK. RIOCH and EDWARD W. DEMPSEY (by invitation). Department of Anatomy, Harvard Medical School, Boston, Mass.

The changes in reflex responses resulting from prolonged tetanic stimulation of the afferent nerve were studied in spinal and decerebrate cats. Observations were made on the following reflexes: ipsilateral flexor, ipsi- and contralateral extensor, and inhibition of the knee jerk and of the contralateral extensor. Stimulation in all cases was maximal. The current was supplied by 60 cycle a.c. through a potentiometer or by a photo-electric cell stimulator with variable frequency. Tests were made to determine that the subsidence of the reflex during continued stimulation was not due to changes in the afferent nerve in the neighborhood of the electrodes.

It was found that the different reflexes varied considerably in their rate of subsidence and in the relation between this and the frequency of stimulation.

The enhancement of a standard test reflex by the prolonged stimulation persisted in many cases after the total subsidence of the prolonged reflex response. Still later the test reflex could be evoked essentially as in a preceding control period. After-discharge of the test reflex, however, was almost invariably diminished and was frequently abolished by the prolonged stimulation.

In spinal cats in which an ipsilateral extensor reflex was present the knee jerk was inhibited (not occluded) during the first few seconds of the prolonged stimulation. The knee jerk then reappeared, small and irregular, and the ipsilateral extensor slowly declined. After stimulation for several minutes the knee jerk recovered almost completely and at this time cessation of the prolonged stimulus was followed by a slowly developing rebound contraction which summed with the knee jerk. The testing reflex varied considerably in these experiments, but its ipsilateral extensor component was diminished or absent during the period of rebound contraction.

*Some effects of androgens and estrogens on the reproductive organs and hypophyses of adult male rats.* WARREN O. NELSON. Wayne University, College of Medicine, Detroit, Mich.

The administration of oestrogenic hormone (oestrone or oestradiol) of 500 or more international units per day for 23 days had an effect upon the testis similar to that produced by hypophysectomy. Sperm production ceased and the sex-accessory glands were reduced to a size approximating that seen in hypophysectomized rats. The animals regularly lost

weight (about 40 grams) during the injection period and their pituitaries showed the usual weight increase and chromophilic degranulation.

The simultaneous administration each day of 500 to 1000 international units of oestrogen and 1 mgm. of androstane dione, a substance previously shown to be very effective in maintaining spermatogenesis in hypophysectomized animals, resulted in the maintenance of normal spermatogenesis in the intact rat. Sex accessories were normal and the animals maintained their body weights or gained slightly. The characteristic effects of oestrogenic hormone on the pituitary were strikingly modified. Only slight weight increases occurred and the degree of degranulation of the chromophilic cells was markedly reduced.

The administration of 1 mgm. androstanedione daily for 23 days resulted in an actual increase in testis size. Although slight in some cases the increase was regular and probably significant. The seminal vesicles were approximately normal in size, but the prostates showed a regular increase. Hypophyses were not enlarged and no significant cytological alterations could be detected. All animals showed a progressive weight increase of of 40 to 60 grams during the period of treatment.

In hypophysectomized males the administration of oestrogenic hormone in the above amounts frequently results in death of the animal. Weight loss is excessive and the reproductive organs show more severe damage than in untreated hypophysectomized animals. The addition of androstane dione to the treatment has resulted in a much better survival and a partial protection against the excessive weight loss and the testicular damage caused by the combined treatments of hypophysectomy and oestrone administration. As previously reported, androstane dione alone in the hypophysectomized male results in the maintenance of normal spermatogenic function.

*Ketogenic and diabetogenic action of anterior pituitary extracts.* A. H. NEUFELD (by invitation) and J. B. COLLIP. Department of Biochemistry, McGill University, Montreal, Canada.

The ketogenic and diabetogenic action of anterior lobe extracts may be due to the same principle or each may be due to a separate principle. Our experiments suggest but do not show conclusively that one substance only is involved. Using the ketonemia of treated fasted rats as an index of ketogenic activity of anterior lobe preparations, the ketogenic activity of various extracts has been determined. The most significant finding in this study has been the demonstration of definite ketogenic activity in a simple aqueous extract (pH 5) of dehydrated and defatted anterior lobes after 15 minutes at pH 10 in a boiling water bath. A similar positive effect was obtained in adrenalectomized rats although our results on ketonuria in such animals agree with those of Long and Lukens, Fry and others. Some positive evidence that the diabetogenic substance is present in this heat treated extract will be presented.

*Alteration in the time relations of the various phases of the respiratory cycle by carbon dioxide.* HAYDEN C. NICHOLSON and SIDNEY SOBIN (by invitation). Department of Physiology, University of Michigan, Ann Arbor.

The respiratory cycle is customarily divided into three phases: 1, inspiration; 2 the first phase of expiration, when air is leaving the lungs, and

3 the second phase of expiration, the expiratory pause. Hammouda and Wilson have stated that the sum of phases 1 and 2 is relatively constant under various conditions, variations in respiratory rate resulting almost entirely from variations in the duration of the expiratory pause. For the sake of convenience we have referred to the sum of phases 1 and 2 as the period of inspiratory stress, assuming that the duration of phase 2 is dependent to some extent at least on a persistence of inspiratory activity.

Carbon dioxide may cause an acceleration of respiration, a slowing, or be without effect upon rate. When carbon dioxide caused a slowing, the slowing occurred mainly as a result of a lengthening of the period of inspiratory stress. In many cases the expiratory pause actually decreased.

When carbon dioxide caused an acceleration, in about half the cases the period of inspiratory stress increased in duration. In only about thirty per cent of the cases did it definitely decrease. The expiratory pause was invariably shortened.

In many cases in which carbon dioxide did not alter the rate of respiration, there occurred a marked increase in the duration of the period of inspiratory stress with an equal decrease in the duration of the expiratory pause.

Whether carbon dioxide caused an acceleration or a slowing of respiration, it almost invariably increased the speed with which the inspiratory movement was carried out. Since carbon dioxide in a majority of cases increased the duration of the period of inspiratory stress, this increase occurred solely as a result of a marked increase in the duration of the first phase of expiration.

It appears that, contrary to the views of Hammouda and Wilson, alteration in the duration of the first two phases of the respiratory cycle is a very important factor in variations in respiratory rate.

*The effect of anoxemia on the absorption of glucose by the small intestine.* David Northup, Edward J. Van Liere and Clark K. Sleeth (by invitation). Department of Physiology, West Virginia University, Morgantown.

It has been previously shown that water and isotonic sodium chloride solution absorption from the small intestine are markedly affected by anoxemia of various degrees.

In the present study isotonic glucose solution was placed in an intestinal loop made up of the entire ileum and most of the jejunum. Pairs of dogs of the same weight were used; the loops were measured to be of the same length. One dog was placed in a low-pressure chamber for inducing anoxemia, the other kept at atmospheric pressure as a control. After one and a half hours the loop was removed; its contents were measured and analyzed for glucose content.

It was found that the absorption of glucose from isotonic solution was not affected by any degree of anoxemia used (the most severe degree was an oxygen partial pressure of 53 mm. Hg), but that the amount of fluid remaining in the intestinal loop was altered to a marked extent. At atmospheric pressure a considerably higher percentage of glucose than of fluid was absorbed—i.e. the solution was more dilute with respect to glucose than when it was introduced into the loop. The lower degrees of anoxemia somewhat reduced this effect, and while the absorption of glucose proceeded at the same rate as at atmospheric pressure, relatively more water was apparently absorbed along with it. At an oxygen tension of 80 mm.

Hg glucose and fluid were absorbed at nearly the same rate. At 53 mm. Hg the absorption of fluid is again apparently less than that of glucose, and the situation is about the same as at atmospheric pressure.

These changes in the amount of fluid recovered at various degrees of anoxemia are believed to be due to effects on the secretion rate of the intestinal glands.

*The melanophore hormone and a thermostable metabolic stimulant in pituitary extracts.* D. K. O'Donovan (by invitation) and J. B. Collip. Department of Biochemistry, McGill University, Montreal, Canada.

Further investigation of the thermostable calorigenic principle present in pituitary extracts (previously described) was undertaken. It was demonstrated in anterior and posterior lobe extracts, also in pituitary colloid and pars intermedia extracts Doctor Astwood, Johns Hopkins Hospital) in amounts approximately corresponding to their melanophore activity. The equivalent of 10 mgm. of pituitary colloid produced a significant rise in B.M.R. The active principle was destroyed by prolonged tryptic digestion, and adsorbed by charcoal. Although it resisted the temperature of the boiling water bath for 15 minutes at pH 10–11, there was no conclusive evidence that this treatment increased its activity. On the other hand, alkali potentiation was not found to be an invariable characteristic of our melanophore preparations.

The increased oxygen consumption in rabbits after a single large injection (corresponding to 50,000 melanophore units) may be prolonged for 12 to 24 hours. The associated respiratory stimulation produces a fall in the $CO_2$ combining power (11.2 cc. per 100 cc. blood, average of 8 animals). The R.Q., if estimated more than 6 hours after injection varies from 0.72 to 0.80 if the B.M.R. is increased more than + 10 per cent. The animals were fasted from 2 to 48 hours. The preinjection R.Q. of non-fasted rabbits receiving daily injections showed in some cases values greater than 1.00 (max. = 1.10).

There was no significant change in liver glycogen within 2 hours after intravenous injection of such pituitary extracts under amytal anesthesia.

In conjunction with results simultaneously published by co-workers from this department, the possibility is suggested that a series of effects caused by pituitary extracts are attributable to one active principle. These physiological effects are: (1) the metabolism-raising action apart from thyrotropic action; (2) the ketogenic effect; (3) the anti-insulin effect; (4) the frog melanophore expanding effect.

Variation in dosage and in the physiological condition of the animal may explain anomalous results.

*Continuous observations of arterial and venous blood pH responses to various experimental procedures.* O. S. Orth and A. Schoenenberger (introduced by J. A. E. Eyster). University of Wisconsin Medical School, Madison.

Continuous arterial or venous blood pH determinations have been made by use of a vacuum tube voltmeter and type K, L & N potentiometer in conjunction with glass electrodes with indwelling calomel half-cells. The electrodes were sealed into the side arms of paraffined T cannulae, placed respectively in the common carotid artery and external jugular vein of dogs under nor-amytal or nembutal anesthesia. The animals were heparinized just before releasing the initial flow of blood, which

thereafter proceeded uninterruptedly through the cannulae. By means of a mercury switch alternate readings could be made between the arterial and venous bloods within periods of thirty seconds. Thus with one amplifier pH potential changes in both vessels could be followed and later graphically compared.

Rise or fall in the pH potentials of the two bloods paralleled almost exactly, venous readings being slightly lower than arterial. Although the barbiturates are known to produce an initial lowering of blood pH (by 0.1–0.2) a relatively constant base line was found to be established within a short time after readings began. We were then able to determine blood pH changes to various experimental injections into the blood stream via the femoral vein. Observations could be followed throughout the time of the injection, as evidenced by control injections of equal quantities of isotonic Locke's solution, with no disturbance of resting base line.

Sodium bicarbonate, sodium carbonate, ammonium chloride, sodium nor-amytal, sodium nembutal, picrotoxin, and other substances have been introduced into the circulatory system in varying amounts, concentrations, and rates, with proportionate variations in degree of pH rise or fall and recovery time. Small quantities of weak concentration produced changes of but a few hundredths pH unit; large amounts of the weak concentration, or small amounts of greater concentration produced changes of almost a tenth of a unit. Recovery of acid-base equilibrium required 2–20 minutes.

*The thermal effects of short wave diathermy on bone and muscle.* STAFFORD L. OSBORNE and JOHN S. COULTER (introduced by F. T. Jung). Departments of Physiology and Physical Therapy, Northwestern University Medical School, Chicago, Ill.

This investigation was undertaken by the authors to ascertain: first, whether heat can be produced in bone marrow in living animals by subjecting the leg of a living animal to short wave diathermy, and, second, if the bone marrow is heated, is the temperature of the bone marrow higher than that of the adjacent muscle?

The femur of large dogs was selected for the experiment. Thermocouples were used with a Leeds and Northrup potentiometer to take temperature measurements before and after application of the high frequency current. Acute experiments were performed. Thermocouples were introduced by means of two hard rubber cannulae one at a depth of 1.5 inches into the muscle, and the other introduced into the bone marrow through a drillhole. The thermocouples in the bone and muscle were in the same anterior-posterior plane to locate them the same distance from the source of heat.

Treatment was given for 20 minutes. Wave lengths of 6, 12 and 24 were used. The high frequency electric field and electromagnetic field was employed as the source of energy, the former by means of air spaced electrodes and the latter by means of the cable pancake coil.

Eighteen experiments were performed. Final terminal temperatures and average temperatures of bone marrow and muscle are recorded. Bone marrow temperatures while elevated did not reach that of muscle. When tissues were damaged the heat gradient was shown to be from skin to bone marrow. Higher temperatures were secured with the electromagnetic field than with the high frequency electric field. No specificity for the

various wavelengths used was found. Muscle heated to a greater extent than did bone. 1. The local application of short wave diathermy to the thigh increases the temperature of the bone marrow, and, of course, the temperature of the bone marrow is increased more than that of the rectum or general body temperature. 2. During the application of short wave diathermy to the limb of a living animal the thermal gradient is from the periphery to the interior. The temperature of the muscle is higher than that of the bone marrow. Similarly, if injury accidentally occurs, the degree of injury decreases from the periphery to the interior. 3. No true evidence was obtained indicating that living tissues manifest a specific thermal response to short waves of different lengths.

*Simultaneous determinations of the body water available for dilution of exogenous urea and sulfanilamide in unanesthetized dogs.*[1] E. E. PAINTER (introduced by Magnus I. Gregersen). University of Maryland School of Medicine and College of Physicians and Surgeons, Columbia University.

In separate experiments with urea and sulfanilamide given intravenously, the time-concentration curves for blood indicate that equilibrium between blood and tissues is approached about an hour after injection. Beyond this point the disappearance rate is constant for several hours (4–5). For exogenous urea (200–350 mgm. per kgm.) the disappearance rates ranged from 9 to 15 per cent per hour, for sulfanilamide (55–65 mgm. per kg.) from 6.0 to 9.1. On the assumption that linear extrapolation back to the time of injection gives the concentration which would be obtained if distribution were complete and none of the substance had been excreted, the "available water" calculated from 8 urea experiments averaged 63.0 per cent of the body weight and from 5 experiments with sulfanilamide 67.0 per cent. Both substances are therefore distributed in approximately the same volume.

This conclusion is confirmed by injecting measured amounts of urea and sulfanilamide simultaneously. The dilution (corrected for disappearance as above) is then within the limits of experimental error, the same for both. In 6 experiments the "available water" calculated from urea ranged from 62 to 68 per cent of the body weight, the average 65.3; for sulfanilamide the range was 63 to 69, the average 66.0. It is probable that these values represent total body water, since the relative amount of sulfanilamide or urea taken up by the tissues per unit weight is with minor exceptions directly proportional to their water content (Marshall). Furthermore, the figures agree closely with the total body water determined by desiccation (literature).

If the calculation of "available water" is based on a single blood sample taken during the second hour (when distribution is presumably complete) and on the amount of sulfanilamide or exogenous urea remaining in the body (total injected minus that recovered in urine), the values are 2–3 per cent higher than those obtained from the disappearance curves.

*Splenic contraction and the artificial fever polycythemia in dogs.* LAURENCE L. PALITZ (introduced by Arno B. Luckhardt). Physiological Laboratories, University of Chicago, Ill.

[1] Aided by grants (to M. I. Gregersen) from the Committee on Grants-in-Aid, National Research Council and The Rockefeller Foundation.

It has been repeatedly shown that in conditions causing a contraction of the spleen, there is a subsequent absolute increase in red blood cells in the circulation. It is therefore significant that in interpreting results of changes in blood constituents, the spleen should be taken into account.

Using the method described at a previous meeting (Palitz and Morse, Am. J. Physiol. **116:** 118, 1936) for determining splenic volume changes in the unanaesthetised dog, it was observed that with artificial fever produced with high frequency currents (G. E. Inductotherm), there was a definite prolonged contraction of the spleen.

To determine whether this contraction was significant in causing the polycythemia induced with artificial fever therapy, normal and splenectomized dogs were given Inductotherm treatments, raising the rectal Temperature from 2 to 4 degrees. Samples of blood were removed before inducing the fever, a half hour after, an hour after and when the temperature had returned to normal. Red cell counts, hematocrit and serum protein (total nitrogen, Van Slyke) determinations were made. All experiments were performed on intact, unanaesthetized dogs.

In the normal animals, a typical experiment showed a red cell increase of 26 per cent, rise in cell volume of 15 per cent, and a serum protein increase of 12.5 per cent. With the spleen removed, there was no significant rise in either the formed elements of the blood or in the protein.

In an effort to explain the lack of protein increase in the splenectomized animals, dogs were splenectomized in acute experiments, concentrated blood from another dog was injected at intervals (after taking hematocrit and protein determinations of donor's blood), thus attempting to simulate experimentally a splenic contraction. Cell counts, hematocrit and serum proteins were taken after each injection. Preliminary experiments show a definite increase in serum protein coincident with the rise in hematocrit and red cells caused by the injection. This seems to indicate that in the normal dogs, the red cell increase, due to contraction of the spleen, caused a concentration of serum, possibly in an effort to maintain a constant blood volume.

*The influence of thyroparathyroidectomy and gonadectomy on the skeletal structure of albino rats.* Mary C. Patras (by invitation), R. D. Templeton and I. F. Hummon (by invitation). Departments of Physiology and Radiology of Loyola University, School of Medicine, Chicago, Ill.

This report summarizes results obtained on 73 albino rats which were weaned at the age of 21 days and maintained on the stock diet which consists of Fox Chow *ad libitum*, with bread, meat and cabbage once weekly. Littermates of the same sex varying in age from 65 to 75 days were then divided into 4 groups. One group (11 females and 12 males) was kept for control; a second (12 females and 7 males) was gonadectomized; the third (10 females and 8 males) was thyroparathyroidectomized, while the fourth group (7 females and 6 males) was both gonadectomized and thryoparathyroidectomized.

One year after the operations all animals were sacrificed; certain bones were cleaned of all flesh, measured and mounted on cardboard. Thus arranged we were able to obtain an x-ray film of all bones with the same exposure.

The femurs and scapulae of the castrated females were longer than

thoseof the control females, while the corresponding bones of the castrated males were slightly shorter than their controls. Measurements of these bones in both sexes of the thyroparathyroidectomized groups revealed them to be shorter than their respective controls.

Gonadectomy was found to have the same effect upon the measurements of the femurs and scapulae of thyroparathyroidectomized animals that it had upon normal animals. The length of these bones was increased in the females and decreased in the males by the gonadectomy.

A study of the x-ray film revealed the normal characteristic pattern of the skull bones not to be impaired by gonadectomy while thyroparathyroidectomy greatly disturbed this pattern giving a mottled appearance. The density of the skull plates was considerably increased in the thyroparathyroidectomized group. The suture lines, on the other hand, were more dense in the control animals. Gonadectomy did not seem significantly to alter the skull bone pattern of either normal or thyroparathyroidectomized animals.

*The relation between chloride absorption and deuterium oxide absorption in the intestine.* HOWARD C. PETERS (by invitation) and MAURICE B. VISSCHER. Department of Physiology, University of Minnesota, Minneapolis.

Ingraham, Peters, and Visscher (J. Phys. Chem. **42:** 141, 1938) showed that absorption of chloride from isotonic solutions containing half isotonic sulfate can be described by the equation, log $(C/C_o)$ = $(R_i/D)$ log $(V/V_o)$ (1), where C and V are concentration and volume at any time, $C_o$ and $V_o$, their initial values, $R_i$, the hypothetical rate of water secretion, and D, the rate of volume decrease. The equation was developed from the theory that the absorption of water "washes" chloride out of the gut without changing its concentration while a secretion of fluid practically free of chloride occurs. The agreement between experiment and theory depended on the choice of a single arbitrary constant $R_i$. In another form of (1), log $(CV/C_oV_o)$ = $(R_o/D)$ log $(V/V_o)$, $R_o$ is the hypothetical rate of water absorption. If no assumptions are made as to the nature of the arbitrary constant, log $(CV/C_oV_o)$ = $(K_{Cl^-}/D)$ log $(V/V_o)$, where $K_{Cl^-}$ is the volume cleared of chloride in unit time.

We have used deuterium oxide in an attempt to show that $K_{Cl^-}$ is actually equal to the rate of water absorption. If $D_2O$ were a perfect indicator of active water movement, $K_{D_2O}/K_{Cl^-}$ would equal one. Actually diffusion of $D_2O$ must occur whether water is moved or not. It can be shown that $K_{D_2O}/K_{Cl^-}$ = log (fraction $D_2O$ unabsorbed)/log (fraction $Cl^-$ unabsorbed). In our experiments on absorption from intestinal loops in anesthetized dogs, $K_{D_2O}/K_{Cl^-}$ approaches one as $K_{Cl^-}$ increases. This apparently represents an approximately constant diffusion error which becomes unimportant at high chloride absorption rates. $K_{Cl^-}$ therefore appears to be equal to the rate of active water movement as required by the theory.

| | EXPERIMENT 1 | EXPERIMENT 2 | EXPERIMENT 3 | EXPERIMENT 4 |
|---|---|---|---|---|
| $K_{Cl^-}$, cc./min | 1.43 | 2.56 | 3.96 | 4.24 |
| $K_{D_2O}/K_{Cl^-}$ | 2.10 | 1.70 | 1.35 | 1.01 |

*Observations on the changes of total filterable and Ca++ concentration following the intravenous injection of calcium chloride.* JAMES G. PHILLIPS and PARKE H. WOODARD (introduced by O. O. Stoland). Department of Physiology, University of Kansas, Lawrence.

It has been shown that there is a sudden and marked rise in the total serum calcium following an intravenous injection of a soluble calcium salt in dogs. The increase and return to normal depends upon the amount injected. Woodard and Lands have shown that apparently the calcium diffuses rapidly into the tissue until some equilibrium is reached with that in the blood stream.

McLean and Hastings have shown that the Ca++ concentration in blood could be determined by the frog heart method. Since this method has a relatively limited working range, they further showed that the Ca++ determination was proportional to the concentration of total serum calcium and the plasma proteins.

To determine whether this relationship existed in high concentrations of calcium, the Ca++ concentration of filtrates obtained by a method as described by Nicholas was performed. Since no protein appears in the filtrate, dilution of the latter would not interfere with the mass action law,

$$\frac{(Ca^{++}) \times (Prot.^{=})}{(Ca\ Prot.)} = K$$

From this equation, McLean and Hastings proposed a method of determining Ca++ concentration from the total protein and total calcium concentrations of blood. To see if this could be applied to abnormally high concentrations of calcium in the blood, calcium was added to samples of dog blood to which an anticoagulant (sodium polyanethol-sulfonate) had been added. The results obtained by diluting the filtrate and the plasma agreed closely with the calculated Ca++ concentration.

Dogs were then injected intravenously with a soluble calcium salt and the changes in Ca++ concentrations were compared to changes with total. It was found that the Ca++ concentration was proportional to the total calcium.

*Observations on hypophysectomized rats.* R. A. PHILLIPS and H. GILDER (introduced by J. C. Hinsey). Department of Physiology, Cornell University Medical College, New York City.

The intestinal absorption of dextrose in the albino rat, by the Cori technique, was found to be about 190 mgm. per 100 gram body weight per hour in male rats 8 weeks old. Rats hypophysectomized at this age and studied 5 weeks later were found to have intestinal absorption coefficients of 100 mgm. to 125 mgm., approximately the same as their unoperated mates. However, as reported previously, the hypophysectomized animals did not deposit the absorbed dextrose as muscle and liver glycogen to the same extent as did the normal rats.

The administration of phlorhizin to the hypophysectomized rats decreased the intestinal absorption of dextrose to the range of the xylose absorption in normal rats, hence indicating that the "diffusion" portion of the absorption mechanism is not at fault.

The per cent of creatine in the muscle of the hypophysectomized rat was found to be about the same as that of their litter mates, and definitely lower than that of their weight mates.

The per cent of the total body weight that is muscle (wet weight) is about 3 per cent less in the hypophysectomized rat, while the per cent that is liver is very nearly the same.

These studies of intestinal absorption in the normal and hypophysectomized rat would indicate that the proper comparison of carbohydrate studies should not be on the weight-mate basis. Since the per cent of neither muscle nor liver in the hypophysectomized rat is increased, these observations lend further support to the evidence that the hypophysectomized rat has a deficiency in his ability to deposit absorbed glucose as glycogen.

*The effect of anoxemia on gastric secretion.* ARTHUR D. PICKETT (by invitation) and EDWARD J. VAN LIERE. Department of Physiology, West Virginia University, Morgantown.

The effect of various degrees of anoxemia on gastric juice was studied on dogs provided with a Pavlov pouch. Anoxemia was produced by placing the animals in a large respiratory chamber from which air could be withdrawn faster than it could enter. Adequate ventilation was provided and there was no accumulation of carbon dioxide.

At oxygen percentages of 12.88 and 10.56 corresponding to 444 and 382 mm. Hg pressure and to altitudes of 14,000 and 18,000 feet respectively, the amount of free and combined acids secreted were distinctly depressed. The period of gastric secretion, however, was definitely prolonged. This was to be expected since anoxemia prolongs gastric emptying time. The results suggest that the total amount of gastric juice secreted was somewhat depressed at severe degrees of anoxemia. This was especially noticeable during the first few hours of the experiment.

*Combined lesions of the decussations of the pyramidal tracts and of the medial lemniscus with bilateral vestibular destruction.* F. H. PIKE. Department of Physiology, Columbia University.

The arguments of Goltz and of von Monakow against the concept of "centers" can no longer be disregarded. Instead, we have the problem of finding the various parts of motor mechanisms, which are sometimes widely dispersed, lesions of one or more parts of which entail motor disorders. Hughlings Jackson and Fritsch and Hitzig emphasized the rôle of the afferent impulses in motor control. It has seemed desirable to investigate the amount added to the picture of combined median longitudinal incision of the decussations of the cortico-spinal tracts and of the medial lemniscus by subsequent extirpation of both vestibular portions of the ears.

The immediate effects of the bilateral vestibular lesions, made about a year after that of the pyramids and lemniscus, were less severe than in control animals. Nystagmus was neither marked nor long continued. The cat resumed walking within three days, and there were none of the violent rolling movements often seen in controls. The general motor facility of the cat is worse six months after the vestibular operation than it was before. The gait is more unsteady, with pronounced swaying, and the facility for climbing is reduced. All the movements are slow and deliberate. The effect does not seem so much to be a matter of new symptoms as of increased gravity of those previously present. Touch seems unaffected, and the animal rubs against an extended hand, but falls to one side when the hand is suddenly removed. Jumping and stepping

across an intervening space from one table to another are seriously affected. Appetite is good and the coat is kept clean. There are no forced movements suggestive of cerebellar injury, nor do the symptoms at all resemble those following lesion of the inferior olive.

We may recall Goltz's statement that if recovery, complete or partial, follows a lesion of any structure, that structure cannot be the sole mechanism for the function which was first disturbed. What other structures are concerned in the functions persisting can be determined by experiment alone.

*Ascorbic acid and glutathione in the rabbit pituitary during pregnancy.*[1] GREGORY PINCUS, F. L. MAYNARD (by invitation) and M. ROSS (by invitation). The Biological Laboratories, Harvard University, Cambridge, Mass., and The School of Agriculture, Cambridge University, England. (Read by title.)

In the course of an investigation of the control of ovum growth and implantation in the rabbit the pituitaries of 22 rabbits sacrificed at various stages of pregnancy were titrated for ascorbic acid with the Tillman reagent and for total iodine reducing capacity using $KIO_3$ after the method of Woodward and Fry.[2] The data are presented in the following table:

| DAYS PREGNANT | NUMBER OF RABBITS | $KIO_3$ | ASCORBIC ACID | ASCORBIC ACID MINUS $KIO_3$ VALUE |
|---|---|---|---|---|
| | | *cc. per gram* | *mgm. per gram* | |
| 0 | 5 | 9.44 | 2.42 | 1.46 |
| 1 | 3 | 10.93 | 1.65 | 0.56 |
| 2 | 2 | 11.32 | 1.84 | 0.41 |
| 3 | 3 | 10.10 | 1.68 | 0.67 |
| 6 | 3 | 7.00 | 1.89 | 1.19 |
| 9 | 2 | 6.40 | 1.88 | 1.24 |
| 13 | 2 | 9.72 | 2.66 | 1.70 |
| 26 | 2 | 6.88 | 2.25 | 1.58 |

Ordinarily GSH concentration would be determined by subtracting the $KIO_3$ equivalent of the ascorbic acid from the total $KIO_3$ value, the remainder being attributed to GSH. Since we found that 1 mgm. of ascorbic acid reduced 10 cc. of our $KIO_3$ solution it is obvious that a negative value for GSH would be had in each instance. Assuming as a rough approximation that all the iodine reducing capacity is due to ascorbic acid (the nitroprusside reaction is very weak or absent) we obtain the data of the last column of the table which may be taken to give an index of the amount of some compound which decolorized the Tillman reagent but scarcely reduces the iodine. It is remarkable that these data (and the ascorbic acid concentrations to a lesser extent) parallel with great fidelity the course of change in pregnancy of ovulation producing substance in the rabbit pituitary reported by Hill.[3] Erzthioneine may represent the presumable compound since it decolorizes Tillman's reagent in greater amount than would be expected from its iodine reducing action and it is present in the pituitary in appreciable quantities.

[1] Aided by a grant from the Josiah Macy Jr. Foundation.
[2] Woodward and Fry. J. Biol. Chem. **97**: 465, 1932.
[3] Hill. J. Physiol. **83**: 1934.

*Evidence for chemical control of "spontaneous" activity of isolated ganglia.* C. Ladd Prosser. Clark University, Worcester, Mass.

In deafferented abdominal ganglia of the crayfish "spontaneous" activity of many neurones is observed. The neurones discharge rhythmically for varying durations and there is no synchrony among the individual units. The activity originates in the non-axon portion of the neurones and is not due to injury. (Prosser, J. Cell. and Comp. Phys. 1934, 4, 185.) The effects of drugs, respiratory poisons, and changes in salt balance upon this "spontaneous" activity have been investigated.

Acetylcholine and eserine in moderate concentrations have no effect upon the "spontaneous" activity; eserine has an injurious stimulating action in high concentrations. Nicotine and adrenalin initially stimulate and later depress activity. Insulin excites in low and depresses in high concentrations; this suggests a specific effect of insulin upon neurones.

Cyanide in dilutions of 1 in $10^4$ has an initial stimulating effect; many cells are stimulated to discharge a few times at high frequencies. Activity then declines to zero. Recovery from this depression occurs on washing with saline.

Variations in the Ca/K ratio of the perfusing saline by varying the calcium when the potassium is constant have no effect, while variation of the potassium with the calcium constant produces striking results. Increase in the potassium from 0.75 gm./l. decreases the "spontaneous" activity so that at 1.5 gm./l. this activity quickly and reversibly stops. Decrease in the potassium content increases the "spontaneous" activity so that at 0.1 gm./l. the discharge is increased several fold. Most of the change appears as an alteration in the number of active units and not in the frequency of discharge of single units. The height of the axon spikes is also reduced with the higher amounts of potassium. It is suggested that the normal "spontaneous" activity depends upon a potassium gradient across the active membranes of the cell bodies and that this gradient is built up by an oxidative mechanism.

*The action of drugs on intestinal motility.* C. B. Puestow. Department of Surgery, University of Illinois, Chicago. (Motion picture.)

A series of operations on an adult female patient resulted in a preparation which permitted direct observation of the motility of the small intestine and right colon. The cecum and ascending colon were isolated from the remainder of the gastro-intestinal tract, the continuity of which was reestablished by an anastomosis between the terminal ileum and transverse colon. Through a colostomy in the mid-ascending colon the cecum and much of the right side of the large bowel protruded as an everted hernial sac which contained several loops of small intestine. Motility of the right colon could be directly observed and action of the small bowel could be seen easily through the single thin layer of colon which covered it. An opposite or contrary motility was found to exist between the large and small intestine. When the small bowel was vigorously motile the right colon was inactive; when the right colon was contracting the small intestine showed little or no motility. At times both the large and small bowel were quiet, but simultaneous vigorous peristalsis of the large and small intestine was never seen.

Studies were made of the effect of many drugs upon intestinal motility. Those drugs, such as morphine, paragoric, eserin, acetylcholin and prostig-

min, which stimulate small bowel peristalsis inhibit or paralyse the right colon. This can explain the constipating effect of the opiates. Pituitrin and pitressin which cause vigorous contractions of the right colon have an inhibitory influence on small bowel motility and cause a loss of muscle tonus. Motion pictures have been obtained which demonstrate these responses.

*The rôle of the vagus in the regulation of the pyloric sphincter and adjacent portions of the gut, with especial reference to the process of gastric evacuation.* J. P. QUIGLEY and I. MESCHAN (by invitation). Department of Physiology, Western Reserve University, Cleveland, O.

A series of investigations performed with the tandem balloon method on normal unanesthetized dogs has shown that a number of dietary factors influence motility *similarily* in the pyloric antrum, pyloric sphincter and duodenal bulb. Among the substances investigated, the following (in descending order of effectiveness) depress the motility in all three regions; fats, fatty acids, dextrose, soaps, dilute mineral acids, hypertonic solutions, glycerol and hypotonic solutions. These substances are effective only when placed in the upper intestine. They would retard gastric evacuation chiefly through their depression of antral motility and in spite of a simultaneous inhibition of sphincter tone and motility.

In studies on chronic double vagotomized animals the depressant action of the substances mentioned above was shown to be greatly diminished or abolished. The obvious explanation that a vagal reflex is exclusively involved in the normal response is not favored. The available evidence favors the belief that vagotomy releases the control through the sympathetic nervous system which then interferes with the production of enterogastrone or decreases the sensitivity of the pyloric region to enterogastrone.

Following double vagotomy, gastric evacuation time remains essentially normal. Although propulsive contractions in the pyloric antrum are fundamentally depressed, compensation obtains for the disappearance of the normal inhibitory influence of food in the intestine permits this motility to persist practically without interruption.

*The rate of utilization of vitamin A and carotene by normal and diabetic dogs.* ELAINE P. RALLI and ARTHUR C. PARIENTE (by invitation). The Laboratories of the Department of Medicine, New York University College of Medicine, New York City.

The rate of utilization of vitamin A and carotene in normal and diabetic dogs with and without the addition of these factors to the diet, has been studied. Four normal dogs were observed as follows; prior to the experimental period, two of the dogs were fed 5 cc. daily of carotene in oil, and two were fed cod liver oil 20 grams daily. After feeding for a long enough time to insure the accumulation in the liver of vitamin A and carotene, a piece of liver was removed under ether anaesthesia, and the vitamin A and carotene content determined. One dog then continued to receive cod liver oil, and one carotene. The feeding of these was discontinued in the other two normal dogs. After a period of 68 to 180 days, another piece of liver was removed and the vitamin A and carotene content again determined. Seven diabetic dogs were treated similarly with the difference that the pancreas as well as a piece of liver was removed at operation.

In two diabetic dogs cod liver oil was continued; in two dogs carotene was continued; in two dogs cod liver oil was discontinued at the time of depancreatization; and in one carotene was discontinued. In two dogs a piece of liver was removed at intervals during the experimental period to observe any changes in the rate of change of the liver vitamin A or carotene. Vitamin A was determined, after extraction, in the Hilger Vitameter, carotene in the photoelectric colorimeter. The results were calculated on the basis of the daily rate of gain per gram of liver tissue. The normal dog fed cod liver oil gained more vitamin A than the diabetic. When the feeding of cod liver oil was not continued, the normal dog did not utilize as much vitamin A as the diabetic. When carotene was fed the normal dog, the liver vitamin A increased, not as much however as with cod liver oil, and the liver carotene also increased. The diabetic dog, similarly treated, lost both vitamin A and carotene from the liver. When carotene was not fed to the normal there was practically no change in the liver vitamin A or carotene. The diabetic animal showed a loss of both A and carotene. Liver lipids were done on all animals and the results are considered from this aspect.

*The effect of n-butyl alcohol—Ringer solutions on the alpha excitability of muscle.* ROBERT RAMSEY (introduced by H. A. Blair). Department of Physiology, Medical School and Department of Zoology, College of Arts and Sciences, University of Rochester, Rochester, N. Y.

The effect of different concentrations of n-butyl alcohol-Ringer solutions on direct current alpha excitability of frog sartorius muscles was studied using the methods and technique developed by H. A. Blair. Changes in the strength duration curves of individual muscles were followed at successive times after addition of the drug and upon recovery. The changes in the curves are expressed in the form of a set of constants calculated from Blair's equation for the kinetics of the excitatory state. In particular a factor $K/h$ where $K$ is a constant expressing the rate of growth of the excitatory state per unit of stimulus and $h$ the unit of threshold which the excitatory state must attain before being adequate is calculated from the approximation

$$R = \frac{kh}{K}$$

where $R$ = the rheobase and $k$ the constant, representing the rate of decay of a unit amount of excitatory substance. In a sense this ratio is a measure of the "stability" of the membrane, for if thought of in terms of ions $K$ would have to have a value sufficiently great to allow accumulation of ions to reach the threshold $h$.

The average result for all concentrations was a more or less transient increase in $k$ and in $R$. In individual experiments particularly at low concentrations $k$ or $R$ may increase or decrease independently. No threshold was found for the effect. Expressed in terms of "stability" of the membrane low concentrations tended either to increase the stability of the membrane or to maintain it at its original value. At high concentrations within the narcotic range the "stability" was very markedly decreased as the result of $R$ increasing to a very much greater extent than $k$. In medium concentrations the "stability" fluctuated considerably depending in part on the rates of change of $k$ and $R$ and on their directions.

Self-excitation of the muscle was frequently found upon addition or washout of the drug when the membrane was very "stable." In like manner the magnitude of the decrease in stability would be a measure of the relative narcosis.

*The $O_2$ consumption of liver and brain slices at different levels of pH.* DAVID RAPPORT and ATTILIO CANZANELLI. Department of Physiology, Tufts College Medical School, Boston, Mass.

The $O_2$ consumption of guinea pig liver and brain slices was determined in a Barcroft-Warburg differential manometer system. The medium used was horse serum, made almost $CO_2$-free by acidification with HCl; then brought to the desired pH by the addition of either HCl or NaOH. The pH was determined by means of a glass electrode (Beckmann pH meter). In the experiments with liver, the control medium was brought to an average pH of 7.23. It was found that there was no appreciable difference in the $O_2$ consumption between pH 7.0 and pH 9.5. Below pH 7.0, the $QO_2$ fell practically as a straight line function of pH to zero at about pH 3.5. At the other end of the scale, the pH began to fall very steeply at pH 10.0 and reached zero at between pH 10.5 and 11.

In the experiments with brain, the slope was somewhat different, there being a fairly sharp optimum at about pH 9.3, the $QO_2$ being then 25 to 30 per cent above that at pH 7.25. Below pH 7.0 the $QO_2$ fell, at first somewhat slowly then sharply to zero at about pH 4. Above pH 9.3, the $O_2$ consumption fell rapidly to zero at about pH 11.0.

The serum itself began to consume oxygen at pH 9.5, the curve rising slowly to about pH 10.8, then very rapidly. At the higher levels of pH, a correction was made for this serum $O_2$ consumption.

*Spectrophotometric evidence of the variability of human hemoglobin.* G. B. RAY and M. M. TAYLOR (by invitation). Department of Physiology, Long Island College of Medicine, Brooklyn, N. Y. (Read by title.)

In spite of the evidence that has been presented to show that the spectral characteristics of oxyhemoglobin are constant, observations made at a wave length not usually studied, i.e., 650 millimicra, indicate that this view is not correct. These studies show definitely that the absorption coefficient ($A_{650}$) per unit concentration is not a constant. The concentration was determined from the oxygen capacity and confirmed by the light absorption at 540 millimicra (shown by Ray and Blair to be stable). The light absorption per millimol varied from 0.69 to 1.12. The bloods (30 in number) seemed to aggregate into three groups but no definite evidence was found to prove that three sorts of hemoglobin existed in the subjects studied. One may equally postulate that two types of oxyhemoglobin exist in any given subject in varying proportions.

*An attempt to measure the rate of development of the fundamental internal force in contracting muscle.* ALFRED C. REDFIELD, ELIZABETH ROORBACH (by invitation) and FRANK M. HULL (by invitation). Harvard University and the University of Mississippi.

If the fundamental internal forces arising in muscle on excitation are delayed in developing external tension as the result of viscous resistance or other causes, the record of the development of tension by the muscle must give an incorrect picture of the rate of development of the internal

forces. By comparing the development of tension when a muscle is first stimulated with the redevelopment of tension after the quick release of the muscle during tetanic contraction, the curve describing the rate of development of the fundamental internal forces may be deduced. It results that the fundamental internal forces develop about twice as rapidly as the external force.

The increased transparency of muscle which accompanies stimulation, according to Nicolai, precedes the development of tension in a similar way. It is suggested that the transparency is an immediate objective manifestation of the fundamental change responsible for the contraction of the muscle.

*The effect of anterior pituitary growth factor on muscular efficiency in the white rat.* C. I. REED. Department of Physiology, College of Medicine, University of Illinois, Chicago.

Myograms on the gastrocnemii of white rats showed predominantly greater amplitude of maximal contraction and lesser duration after prolonged treatment with growth factor (Antuitrin G) than in controls of the same ages. The increased growth rate is not therefore a physiological advantage.

*Electrical activity recorded with microelectrodes from the hippocampus.* B. RENSHAW (by invitation) A. FORBES, and C. DRURY (by invitation). Department of Physiology, Harvard Medical School, Boston, Mass.

The hippocampus (cat; pentobarbital sodium) has been examined because it is an elementary cortex; the axons, cell bodies and dendrites of its main cell types fall into definite strata. Histological examination defines precisely the position of microelectrodes which have been lowered into it with a micromanipulator.

Aside from transient effects manifestly due to stimulation by injury, the recorded potential changes fall into two classes, fast and slow.

1. The fast (axon-like) potentials of 1 to 2 milliseconds' duration have been recorded consistently from the layer containing the pyramidal cell bodies, and not from other strata. Though these spikes show several hundred microvolts between the microelectrode and an adjacent surface electrode, they are not recorded between the same surface electrode and another superficial one at a distance or even between a surface electrode and a second microelectrode a fraction of a millimeter from the first microelectrode. In the predominant phase the microelectrode is always negative to the surface electrode. The fast waves often appear in periods of activity alternating with periods of quiet (30 to 60 seconds' duration). Frequently rapid groups of as many as eight spikes appear in which characteristically the potential of successive spikes decreases and the interval between them increases.

2. The "slow waves" have durations of 30 to 70 milliseconds and voltages up to 200 to 300 microvolts, though they are often inconspicuous or absent. They are variable in duration, magnitude, and wave-form as recorded from a single position of the electrodes. They are detected with both gross surface electrodes and microelectrodes in various positions within the hippocampus. The localizing electrode may become either positive or negative to an indifferent region.

All evidence indicates that the slow waves are not the summations of

numbers of axon-like spikes. No intermediate forms are seen; when both types of activity are recorded together they appear to vary independently.

Electrical stimulation of afferent fibers to the hippocampus has evoked potentials (recorded with relatively large surface electrodes) resembling in some ways responses of the spinal cord to stimulation of dorsal roots.

*Nutritional requirements of pregnant and lactating rats studied by the self-selection method.* CURT P. RICHTER and BRUNO BARELARE, JR. (by invitation). Henry Phipps Clinic, Johns Hopkins Hospital, Baltimore, Md.

For at least a month previous to mating, ten female rats had shown normal growth and regular 4 to 5-day oestrous cycles on a diet which they selected from 11 substances in separate containers: casein, sucrose, olive oil, sodium chloride, dibasic sodium phosphate, calcium lactate, potassium chloride, dried Baker's yeast, cod liver oil, wheat germ oil, and water. These animals gave birth to normal litters and nursed most of them until they were weaned 25 days later.

The changes in appetite for the 11 substances were strikingly similar in the 10 animals. It was observed that the animals voluntarily ingested large amounts of fat; in fact, over half (52.6%) of the calories were furnished by fat. The fat consumption increased during pregnancy and lactation, supplying 65.0 per cent of the calories at the end of lactation. Protein furnished 22.3 per cent of the calories before mating, 28.1 per cent at the end of gestation, and 23.8 per cent at the end of lactation. The carbohydrate intake remained on a constant level, and therefore showed a relative decrease in caloric percentage from 25.1 per cent before mating to 11.3 per cent at the end of lactation.

The appetite for sodium chloride, dibasic sodium phosphate, and calcium lactate showed striking increases during pregnancy and lactation. The potassium chloride intake, on the other hand, showed no great change. The appetite for wheat germ oil and cod liver oil also remained essentially the same.

The number of calories increased from 45.3 per day before mating to 59.8 at the end of gestation, and to 118.3 at the end of lactation. This is considerably lower than the daily caloric intake on the McCollum diet. In addition, the daily solid food intake (including the oils) was much lower than on the McCollum diet. The fact that the animals grew and reproduced with as great success as on the McCollum diet, in spite of the lower solid food and caloric intake, is evidence of the efficiency of the self-selection method in the rat.

*Animal cages.* CURT P. RICHTER. Phipps Psychiatric Clinic, Johns Hopkins Hospital, Baltimore. (Demonstration.) Three types of cages used for activity, food and mineral choice, and nest-building experiments.

*Evidence obtained by the use of protamine insulin relating to the question of whether extra insulin is normally required to control post-prandial glycemia.* HENRY T. RICKETTS (introduced by A. J. Carlson). Dept. of Medicine, University of Chicago, Ill.

Diabetic patients of varying degrees of severity but under good control on regular or protamine insulin were given, in the morning or evening of

one day, a dose of protamine insulin which during the entire morning of the next day was demonstrated to maintain the blood sugar at a constant normal level. Several days later in each case, with the patient under identical conditions, the experiment was repeated, this time giving the breakfast allowed by the patient's usual diet. Severe diabetics responded to breakfast with a definitely diabetic type of glycemic curve, whereas the curve in the milder cases was nearly normal. It appears, therefore, that at least in some severe diabetics an amount of available insulin which satisfactorily regulates the endogenous carbohydrate metabolism is unable to cope with even moderate amounts of additional glucose.

Similar experiments were carried out in chronically depancreatized dogs. The behavior of the blood sugar in these animals closely resembled that seen in the severe human diabetics, i.e., a dose of protamine insulin which was adequate to hold the blood sugar at a constant normal level without food entirely failed to prevent hyperglycemia after a regular feeding. There was no evidence of disease of the liver in either patients or animals.

Since these experiments were performed under conditions which were as physiologic as can be obtained in any diabetic organism, it is concluded, contrary to the opinion of Soskin and his co-workers (Am. J. Physiol. **109**: 155, 1934), that post-prandial hyperglycemia in severe diabetes is not controlled without extra insulin. The fact that nearly normal curves were obtained in mild diabetics suggests that these individuals possess enough functioning pancreas to respond to the ingestion of carbohydrate by secreting an extra, though still not optimum, amount of insulin. It further suggests that the blood sugar curve of the normal individual may be regarded as due to a completely adequate pancreatic response.

*The pituitary and sex hormones capable of increasing serum calcium and some conditions affecting their action.* Oscar Riddle and Louis B. Dotti. Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Adequate dosage (during 6–10 days) of mare serum or of gonad-stimulating A.P. hormone increases the serum calcium of normal, hypophysectomized or thyroidectomized adult female pigeons. This hormone is also effective when administered as an unfractionated pituitary extract. A.P. preparations lacking this principle but rich in prolactin, in adrenotropic, or in growth-promoting action have no calcium-raising action in any type of pigeon, dove, fowl, rat or rabbit studied. Ordinary urinary prolans and intermedin are ineffective; thyrotropic hormone is probably ineffective. In immature males and females and in mature males (pigeons, dove, fowl) the serum calcium is unchanged or little changed by dosage during 7–10 days. Less adequate tests made on rats and rabbits have failed to show a calcium-raising action of gonad-stimulating hormone.

The sex hormones show extreme differences in their capacity to increase the serum calcium; here species difference (failure in rabbits) is important though age and sex have relatively little importance. The female sex hormones, theelin and dihydrotheelin benzoate, markedly increase the serum calcium in both sexes of both birds and mammals when given in sufficient amount during appropriate periods. In dogs 3000 r.u. daily for 1–2 days exceeded the effectiveness of dosage for 9 days; in birds the longer period is more effective. We have used normal doves and pigeons; hypo-

physectomized, thyroidectomized or castrate pigeons; hypophysectomized-castrate pigeons; mature and immature fowl; normal and castrate rabbits; normal, hypophysectomized, thyroidectomized and castrate rats; normal dogs. Most pronounced increases (to 28 mgm. per 100 cc.) were obtained with pigeons and fowl (injected for 7 days). Dihydrotheelin and theelol seem relatively less potent than theelin. Progesterone has very low activity. Androstendiol, testosterone and androsterone, even in huge doses (2.0 mgm. daily for 7 days), have shown no effect on the serum calcium of either pigeons or rats.

*Electrical stimulation of the brain stem of cats with unilateral degeneration of the corticofugal fibers.* DAVID MCK. RIOCH and ROBERT S. MORISON (by invitation). Departments of Anatomy and Physiology of the Harvard Medical School, Boston, Mass.

The neocortex on one side, with the exception of fragments of the Gyrus orbitalis and Gyrus genualis, was removed aseptically in a series of cats. Two weeks to six months later at a terminal experiment a similar removal on the remaining side was performed under ether anaesthesia. The anaesthetic was then discontinued and the surface of the remaining brain was explored by electrical stimulation with bipolar silver-silver chloride electrodes, the inter-polar distance being approximately 1 mm. The current was supplied by 60 cycle a.c. through a variable potentiometer, reducing the intensity to 1 to 5 volts. A well-coördinated response, which may be called the "attention reaction," consisting of conjugate deviation of the eyes, turning of the head, and, to a less extent, movement of the ears in the same direction, together with dilatation of the pupils and retraction of the nictitating membranes, was elicited from a wide area rostral and rostro-ventral to the lateral geniculate body on either side. From subdivisions of this area the "attention reaction" was directed up or down in the vertical plane or towards any point in the contralateral arc. Ipsilateral movements were not obtained, and there were no associated movements of the limbs. Movement in the horizontal plane was most regularly evoked and was the strongest. A similar reaction, less complete and only in the horizontal plane, was elicited from the surface of the mesencephalon between the colliculi and along the course of the brachium of the inferior colliculus.

Constriction of the pupils and closure of the contralateral eye resulted from stimulation in the region of the entrance of the optic nerve into the lateral geniculate body.

In further experiments, besides adversive head and eye movements with associated limb movements, various reactions, in part resembling instinctive and emotional behavior, were obtained from stimulation of discrete areas of the subthalamus with the Horsley-Clarke technique.

*An improved device for the study of conduction.*[1] JANE SANDS ROBB and ROBERT C. ROBB (by invitation). Departments of Pharmacology and Anatomy College of Medicine, Syracuse University, N. Y.

Controversy regarding measurements of cardiac conduction velocity has been inevitable because the reading errors in some laboratories have been of the same order of magnitude as the phenomenon studied. Fur-

[1] Grateful acknowledgment is made to the Hendricks Fund.

thermore, 11 of 15 experiments reported by Abramson and Jochim actually support our observation that surface muscle cuts introduce delay (despite the apparently random orientation of their incisions). In the one diagram where they show muscle fibre direction, a nearly transverse cut caused an 0.004,7 second delay, whereas a very oblique incision resulted in only a 0.001,2 second delay, and the average delay for their whole series was 0.002,2 seconds.

Hitherto the accuracy of time measurements has been qualified by string inertia and by the limitations of the optical system which condition paper speed. The Cambridge Instrument Company has designed and built for this laboratory three crystal-torsion galvanometer units, each connected to identical three stage amplifiers. These units are permanently mounted in relation to a battery of three permanently fixed string galvanometers so that all six record simultaneously on a 12 cm. camera. A common time marker, activated by a synchronized motor, intercepts all six tracings and rules the entire paper at 1/100 second intervals. Parallax between the two batteries is less than 1/10,000 second, which is the reading error of the older apparatus. Between the three crystal galvanometers the parallax is less than 1/100,000 second, which is its reading error at a paper speed of 1.6 meters per second. Its initial deflection is apparently instantaneous, but there is a terminal lag due to "electrical soaking of the crystal" producing an asymptotic approach to the new position, requiring 0.000,5 seconds. The amplitude of response is rectilinear for the first cm. above or below its isoelectric level. This apparatus is superior to any hitherto available for the study of conduction in the heart. Records of initial surface negativity obtained with this instrument will be presented.

*Muscle-bundle localization of infarcts in human hearts.*[1] ROBERT C. ROBB (by invitation) and JANE SANDS ROBB. Departments of Anatomy and Pharmacology, Syracuse University, N. Y.

The most reliable method hitherto available for the localization of cardiac infarcts is the anterior-posterior ($T_1/T_3$) classification of Parkinson and Bedford. Experimentally we have found not two but four types of infarct-electrocardiograms; these are derived from the four anatomical and functional subdivisions of the ventricular walls which are the muscle bundles. In 14 of our 25 autopsied medical cases and frequently in experimental animals the $T_1/T_3$ mode of analysis is equally satisfactory. In the remainder of cases the muscle-bundle localization is more accurate. In no case was it misleading.

It was further noted in experimental, and confirmed in clinical, electrocardiograms of infarction that great weight must be attached to aberrations in any portion of the S-wave. In major lesions of the superficial bulbo-spiral muscle the entire interval from the end of S to the beginning of T ($S\text{-}T_1$) in lead I is depressed. In smaller lesions of this muscle there may be a gradual, thickened, asymptotic return of the S-wave to the isoelectric level. Infarcts in this muscle are usually posterior and hence usually confirm at $T_3$ classification. On the contrary, superficial sino- and deep bulbo-spiral muscle injuries resist the $T_1/T_3$ analysis because the S-T intervals are elevated in all standard leads, although not to the same

[1] Acknowledgment is due the Hendricks Fund for the support of this work.

degree in each case. Lastly, deep sino-spiral muscle lesions are commonly described as $T_1$ (anterior) events: in our six cases of this sort the post-mortem localization was twice right antero-lateral, once anterior right-left-and-septal, once anterior-left, once left antero-lateral, and once posterior.

This brief series supplements our experimental studies and suggests that conclusions derived from them are applicable to man.

*The accuracy of indirect blood pressure determination in children.* M. ROBINOW (by invitation), W. F. HAMILTON, R. A. WOODBURY and P. P. VOLPITTO (by invitation). Department of Pediatrics, Department of Physiology and Pharmacology and Department of Anesthesia, University of Georgia School of Medicine, Augusta.

Direct brachial arterial pressures were obtained in anesthetized and unanesthetized children with the hypodermic manometer. Indirect pressures were also determined under basal conditions with cuffs of various widths. The proper size of the cuff was determined by comparison of the direct pressures with simultaneously taken cuff pressures and with the pressures obtained with cuffs of various sizes. The proper size of the cuff for systolic pressure is better estimated from the length than from the circumference of the arm. The proper size of the cuff seems also related to the pulse contour and its change on occlusion of the artery. Children with peripheral pulses need wider cuffs than children with central pulses. The latter occur mainly in infants. Diastolic indirect pressure readings are unreliable. They are usually too high. Diastolic pressures are better estimated (= systolic pressure − 40 mm. Hg) than determined by auscultation. Blood pressures of children change very little with age.

*The effects of single exposures to benzol vapor on the red cells of rabbits.* ELLIS J. ROBINSON (by invitation) and DAVID R. CLIMENKO. The Biological Laboratory, Cold Spring Harbor, N. Y.

Rabbits exposed for two hours to an atmosphere containing approximately 10,000 parts per million of benzol may show immediate reductions in the red cells of the peripheral circulation with falls as great as 25 per cent. The initial fall is usually followed in two or three days by a sharp rise and a subsequent drop which may bring the count to even lower levels than before. It then returns to normal in a time which varies considerable from animal to animal, but is usually about fourteen days. The fall in the count is believed to be the result of cell destruction, for it is never accompanied by any significant change in the plasma protein level. Furthermore, the return to normal is slow and gradual, and is usually associated with a definite reticulocytosis.

Although benzol is known to accelerate the haemolysis of red cells by saponin in in vitro systesm, the cells of rabbits recently exposed to benzol vapor are more resistant to its action than normal cells. It is believed that this indicates that those cells which are most resistant to saponin haemolysis are also most resistant to the destructive processes which take place in vivo.

*Heart rate, exercise and age.* S. ROBINSON (introduced by L. J. Henderson). Fatigue Laboratory, Morgan Hall, Harvard University, Boston, Mass.

The heart rates of 93 males, ranging in age from 6 to 76 years, were

recorded during rest, during a walk, and during exercise that brought on exhaustion in 3 to 5 minutes on a motor-driven treadmill set at an angle of 8.6 per cent and operated at 5.6 km. per hour in the walk and at a rate suited to the capacity of the individual in the harder grade of work.

The heart rates attained during the walk averaged 170 in 8 six-year old boys and 165 in 21 boys from 8 to 15 years old, as compared to 139 in men from 20 to 65 years. Five men above 65 years averaged 150.

The highest heart rate attained during the exhausting work was 211 in a boy of 10, while in men above 60 the highest rate was 167. Between these ages there is a gradual decline in the rate that the heart can attain in severe exercise.

The rate of attainment of the maximal heart rate is slower with advancing age. In boys, 8 to 12 years old, the increase of heart rate during the first minute of work is about 65; in men in the seventh decade, about 40. The difference in deceleration during recovery is in the same direction.

*The development of the chick embryo.* ALEXIS L. ROMANOFF (introduced by J. A. Dye). Cornell University Agricultural Experiment Station, Ithaca, N. Y. (Motion picture.)

The picture is in natural colors. It portrays the physiological manifestations of the life and development of the embryo at daily intervals from a fresh egg to a hatched chick. The various stages of the living embryo are shown by three methods: (1) through the shell by means of transmitted light; (2) through a hole made by removal of a portion of the shell and shell membranes at the blunt end of the egg; and (3) in an embryological dish after a complete removal of the shell and shell membranes. In the production of this picture the normal life and activity of the embryo both *in vivo* and *in vitro* was maintained by the use of a special air-conditioning incubator-stage.

*On the distribution and rate of disappearance of injected histamine in normal and adrenalectomized rats.* BRAM ROSE (by invitation) and J. S. L. BROWNE. McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

Histamine hydrochloride was injected intravenously into adult etherized female rats of the "hooded" strain. At varying time intervals, the animals were again etherized, the blood and tissues removed and their histamine content determined, in the blood by the Code modification of the method of Barsoum and Gaddum, and in the tissues by the method of Best and McHenry. Assays were done on the atropinized guinea-pig ileum, and some of the values for tissues were checked using the blood pressure method in the atropinized cat under ether.

A dose of 0.04 mgm. histamine hydrochloride per gram of body weight was chosen since this was in general well tolerated by the adrenalectomized group which was maintained on 0.9 per cent NaCl. The values in gamma per cubic centimeter or gram as histamine base shown in the table are for individual animals killed at the time indicated.

In both normal and adrenalectomized animals the histamine disappears at a very rapid rate from the blood, even at the end of 15 minutes the distribution is markedly in favour of the tissues. Of the tissues studied the kidney shows the highest concentration. At the end of fifteen minutes only a small fraction of the histamine injected can be recovered in the

| TIME | BLOOD | | KIDNEY | | LUNG | | LIVER | | GLANDS | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Normal | Adrenalectomized | Normal | Adrenalectomized | Normal | Adrenalectomized | Normal | Adrenalectomized | Normal | Adrenalectomized |
| 15 minutes | 13.2 | 20 | 500 | 450 | 64 | 80 | 94 | 150 | 68 | 80 |
| 30 minutes | 12 | 18 | 450 | 380 | 34 | 95 | 12 | 120 | 20 | 90 |
| 1 hour | 3 | 12 | 120 | 250 | 22 | 105 | 10 | 75 | 18 | 102 |
| 2 hours | 0.5 | 10 | 15 | 230 | 10 | 50 | 8 | 50 | 10 | 40 |
| 3 hours | 0.20 | 7 | 2 | 95 | 10 | 17 | 3 | 4 | 30 | 40 |
| 4 hours | 0.07 | 5 | | | | | | | | |

tissues studied. In the normal animals the rate of disappearance of histamine is greatest in the kidney; and the concentration has fallen to normal levels in lung, liver and glands at the end of two hours. After adrenalectomy the rate of disappearance from both blood and tissues is very much slower. The mechanism of destruction of histamine seems to be impaired after adrenalectomy.

*Action of eserine on the superior cervical ganglion.* A. ROSENBLUETH and F. A. SIMEONE (by invitation). Harvard Medical School, Boston, Mass.

The magnitude of the M2 spike of the superior cervical ganglion in response to a maximal test volley from one of the branches of the annulus of Vieussens undergoes the following changes when the test volley is applied at increasing intervals after a maximal conditioning volley from the other branch. The test response first decreases to be minimal when the interval is about 6 msec. It next suddenly increases, first rapidly, then more slowly to become full-sized at intervals of about 30 msec.

After eserine (7 mgm. per kgm.) or prostigmin (1 mgm. per kgm.) the initial decline at short intervals is reduced or abolished. The later increase is slightly delayed.

The initial decline is interpreted as follows. In some cells of the ganglion stimulation of either branch alone only leads to subliminal excitation, but simultaneous stimulation of the two branches produces a discharge. The subliminal excitation caused by the conditioning volley decays progressively, so that the test volley becomes ineffective in an increasing number of these cells as the interval between the two volleys is lengthened.

The later increase of the responses is interpreted as denoting the recovery from refractoriness of elements which discharge in response to fibers in both branches (area of occlusion).

According to these interpretations, eserine retards the decay of the mediating process—as the theory of chemical transmission by acetylcholine predicts. Eserine has also a retarding action on the recovery process of the ganglion cells—a direct effect which lies outside the domain of the theories of synaptic transmission.

*Changes in skin temperature in normal subjects produced by changes in posture.* GRACE M. ROTH (by invitation), MARVIN M. D. WILLIAMS (by invitation) and CHARLES SHEARD. Section in Clinical Physiology and Division of Biophysical Research, The Mayo Clinic and The Mayo Foundation, Rochester, Minn.

Observations on skin temperatures have been used in man as a method for determining changes in blood circulation. The present studies were

made to determine the changes in skin temperatures of the upper and lower extremities with change in posture in normal man.

Observations were made on various regions of the skin of 15 normal subjects in a room in which the temperature and relative humidity were controlled. The subjects were studied under basal conditions. The temperatures of the various regions of the skin were determined by means of thermocouples in a galvanometric circuit so arranged as to permit of obtaining skin temperatures very rapidly and with an accuracy of 0.2°C. (approx. 0.5°C.).

Some of the data were obtained while the subjects were on a tilt table. In these instances the skin temperatures were observed with elevation and lowering of the feet at different angles. Other observations were made under the following conditions: (1) Change of the subject from the horizontal position to a chair and vice versa; (2) change of subject from horizontal position to sitting position on side of bed; (3) Subject lying on the bed with arms perpendicular to the body, arms lowered to the side and the legs elevated about 30 degrees; (4) One arm and leg hanging over the side of the bed with the subject horizontal.

The results showed that, in the majority of cases, elevation of the feet caused a decrease in skin temperatures of the toes, while lowering the feet from the horizontal position caused a rise in skin temperature. The same changes occur in the upper extremities. The physical and physiological factors which affect the character of the changes in the skin temperatures of the extremities, produced by changes in posture, will be discussed.

*The oxygen consumption of rabbit ovaries and pituitaries in anestrus, estrus, and after mating.*[1] BORIS B. RUBENSTEIN. Department of Anatomy and Associated Foundations, Western Reserve University, Cleveland, O.

The oxygen consumption of slices of rabbit antepituitary, ovary, adrenal, liver, and thyroid was determined by the Warburg technique, at 36.6°C. A part of each antepituitary and ovary was studied histologically. The $O_2$ consumption of the antepituitary is maximum in estrus, and during the first two hours after mating; it is least in anestrus and in the last hour before ovulation, at which time, however, the $O_2$ consumption of the ovary is greatest. Absolute values are presented in the table. A few experiments on rats confirm the findings of Victor and Anderson.

These results suggest that preovulative degranulation of the anterior pituitary is chiefly a discharge of stored material, released after the stimulus of mating.

TABLE 1

*The oxygen consumption of rabbit pituitary, ovary, etc.*

| TISSUE | BEFORE MATING | | AFTER MATING | | |
|---|---|---|---|---|---|
| | Anestrus | Estrus | 1-3 hours | 4-6 hours | 7-9 hours |
| | *cmm. per gram per hr.* | *cmm. per gram per hr.* | *cmm. per gram per hr.* | *cmm. per gram per hr.* | *cmm. per gram per hr.* |
| Antepituitary | 430 | 620 | 640 | 570 | 350 |
| Ovary | 320 | 420 | 370 | 430 | 670 |
| Liver | 500 | 440 | 440 | 510 | 420 |
| Adrenal | 320 | 330 | 310 | 310 | 360 |
| Thyroid | 370 | 500 | 510 | 380 | 450 |

[1] This work was in part supported by a grant from the Rockefeller Foundation.

*The distribution of the alpha rhythm in the human cerebral cortex.*[1] MORTON A. RUBIN (introduced by Hudson Hoagland). The Biological Laboratories, Clark University and the Research Service, Worcester State Hospital, Worcester, Mass.

Electro-encephalograms were taken on 15 subjects to determine the distribution of the $\alpha$ rhythm over the cerebral cortex. It was found that unipolar (grid-ground) recording from electrodes on the scalp gave a truer representation of the amount of 10/sec. activity in the cerebral cortex under the electrodes than does balanced (push-pull) recording. The percentage of record occupied by the $\alpha$ rhythm (*per cent time alpha*) was used as the criterion of the amount of $\alpha$ activity.

There were at least two regions of the cortex showing maxima of $\alpha$ activity . . . usually some part of the occipital and frontal lobes. The occipital lobe distribution of *per cent time alpha* was very stable and could be reproduced over long periods of time. The curve describing the distribution of *per cent time alpha* in the frontal lobe, however, was extremely variable, fluctuating over short intervals ($\frac{1}{2}$ hour) and from day to day in a given individual. It was unusual for the occipital lobe to be the only area showing a maximum of $\alpha$ activity. The $\alpha$ rhythms from the two cerebral hemispheres were least synchronized in the back of the head, more perfect unison between the two rhythms being obtained the further anterior one record along the head. It is concluded that the $\alpha$ rhythm may be produced over the entire cerebral cortex, and that it does not originate solely in the occipital lobes.

It is suggested that the marked fluctuation of the $\alpha$ rhythm in the frontal lobes may be due, at least in part, to the influence of thalamo-cortical and hypothalamocortical connections.

A pacemaker common to all cortical areas is postulated to account for the presence of the same frequency (the 10/sec. $\alpha$ rhythm) in functionally and histologically different cortical areas.

*The effect of choline on the liver lipids of depancreatized dogs.* SAUL H. RUBIN (introduced by Elaine P. Ralli). Laboratories of the Department of Medicine, New York University College of Medicine, New York City.

A prophylactic dose of 2 gms. of choline chloride was fed daily to 5 totally depancreatized dogs for periods of from 5 to 22 weeks. At the end of these periods the livers were analyzed for total liver lipids and the following fractions; phospholipid, neutral fat, free and total cholesterol. It was found that a slight degree of fatty infiltration occurred in the livers of these animals, and although the concentration of phospholipids remained normal the values for neutral fat and the iodine number of the total fatty acids were outside the limits previously reported by us for normal dogs on the same diet. All of the animals studied showed a weight loss of from 7 to 49 per cent of the weight before depancreatization. It may be that the slight fatty infiltration observed was a reflection of this loss of weight rather than the result of an inadequacy of the choline dosage.

Lipid and nitrogen determinations were done at intervals on the feces of 2 of these dogs. The results showed that choline feeding had no apparent effect on the intestinal absorption of fat and protein.

[1] Aided by a grant from the Child Neurology Research (Friedsam Foundation).

Frequent examinations with periodide and ammonium reineckate of the urine and aqueous extracts of the feces of one dog showed the presence of only traces of "unbound" trimethylammonium compounds, which is interpreted as an indication of the complete absorption of the choline fed.

*Factors varying the rate of tumor production with ultraviolet irradiation.* H. P. Rusch, C. A. Baumann and B. E. Kline (introduced by W. J. Meek). Department of Physiology, University of Wisconsin, Madison.

The carcinogenic action of ultraviolet light was studied on a group of 747 mice. Sixty-two to 83 per cent of albino mice irradiated for 30 to 60 minutes daily developed ear tumors in 3½ to 9 months. Mice irradiated for long periods daily, developed tumors more rapidly than those irradiated for short periods. Black mice developed a smaller percentage of tumors than albinos and the time required for such production was longer. The rate of tumor production with ultraviolet light also varied in mice kept on five different diets. Tumors were produced most rapidly on a high fat diet (hydrogenated cottonseed oil), whereas on a brain extract or on liver, tumor production was retarded. Addition of 2 per cent cholesterol to the stock diet did not affect the rate of tumor production, although a marked increase in liver fat and cholesterol resulted. In another series of mice all kept on a stock diet, the ears were painted three times a week with various oils and the animals irradiated daily. When mineral oil was used, tumor production was markedly accelerated.

*The effect of certain psychic phenomena on the various phases of gastric hunger contractions.* R. E. Scantlebury (by invitation) and T. L. Patterson. Department of Physiology, Wayne University, Detroit, Mich.

In the progress of experimental work with hypnosis and various other psychic phenomena on the gastric hunger mechanism in man, a marked difference in the reaction was observed dependent on where in the period of active hunger contractions the stimulus was given. A further study of this phase was therefore undertaken.

Studies on the empty stomach of two subjects were carried out by the balloon method. A total of 112 hours of recordings were made, 29 hours of which were normal and various psychic and hypnotic variations were used during the 83 experimental hours. The normal tonal rhythm of the contractions from both subjects fell within the twenty to thirty seconds rhythm.

Division of the active periods into which the stimuli were introduced exhibited different effects. The effect differed when the psychic phenomena were instigated in the first two-fifths of the hunger period and concluded before the period was half over, a second effect was produced when the stimulus fell within the second two-fifths of the hunger period. A third effect was produced if the experimentation was not started until the period of incomplete tetanus had commenced. In the stimuli concluded before the hunger period had passed the two-fifths of its normal length, and in which inhibition occurred, the effect was a lengthening of the time between the individual contractions. The total length of the hunger period was lengthened to compensate for this inhibitory effect and the total number of individual contractions per period remained within range for normal, and the usual tetanus ending occurred. In the second

period, stimulation caused complete inhibition, the contractions stopping abruptly, following a short latent period of two to three contractions, and the stomach passed into quiescence. In the third phase or the period of incomplete tetanus, we were unable to produce any influence on the gastric motility by the use of psychic stimuli either awake or in hypnotic sleep.

*Studies on the secretion of bile and its constituents.* C. ROBERT SCHMIDT and J. M. BEAZELL (introduced by A. C. Ivy). Department of Physiology, Northwestern University Medical School, Chicago.

Bile was collected continuously under slight negative pressure from bile fistula dogs. The application of this principle eliminates certain experimental variables due to temporary partial or complete obstruction or to ascending infection. Over long periods, on any given regime, the daily variation in volume and bile salt output is not more than ±8 per cent. The method permits the quantitative collection of hourly samples of bile; with appropriate analyses the secretory curves of bile secretion and its more important constituents have been determined.

When a standard mixed meal was fed and no bile returned to the intestine the secretory response of the liver was greatest during the second postcibal hour (volume increased 84 per cent; bile salt output increased 115 per cent). When bile of known composition was returned to the intestine during the first three hours following the meal, the maximum response occurred during the second and third postcibal hours (volume increased 300 per cent; bile salt excretion increased 1500 per cent).

Quantitative data dealing with the enterohepatic circulation of bile-salts, cholesterol and bile pigment will be presented.

*A comparative study of oxygen consumption, respiratory quotient and activity during the metabolism measurement of the fasting albino rat.* EDWARD L. SCHWABE (by invitation) and FRED R. GRIFFITH, JR. University of Buffalo, N. Y.

A statistical analysis of 500 rigidly controlled metabolism measurements on the 24-hour fasting albino rat gives a mean respiratory quotient of 0.755 with a range of 0.660–0.850.

An increase in animal activity is accompanied by a corresponding increase in oxygen consumption per unit of body weight, but has no effect on the nature of the foodstuffs burned, as revealed by the unaltered R. Q. and the uniformity of the coefficient of variation for the several degrees of activity. Likewise, prolonged exposure to cold previous to the metabolism determination, while it increases oxygen consumption, does not appreciably modify the respiratory quotient.

*Observations on the blood sugar level before, during and after hunger periods in humans.* W. W. SCOTT (introduced by A. B. Luckhardt). Department of Physiology, University of Chicago, Ill.

The blood sugar level and the state of motility of the empty stomach were recorded on *normal humans* during a post-absorptive period to determine any causal relationship between the blood sugar level and hunger contractions.

Determinations of the blood sugar level were made before, during and after "hunger periods" by the method of Miller and Van Slyke. Hemoglobin concentration was determined on the majority of samples. The

conventional balloon-water manometer method of recording hunger contractions was used.

In a series of 12 experiments on 6 human subjects, the blood sugar values with very few exceptions ranged between 85 and 95 mgm. per cent. This indicates that the blood sugar level is unaltered by states of quiescence or motility of the stomach. The blood sugar level during incomplete tetanus was identical with that during absolute quiescence before or after the hunger period. At no time were there any consistently low (or high) values.

No causal relationship between the blood sugar level and hunger contractions was observed.

*The absorption of insulin from intestinal loops as influenced by phenolic compounds.* ROBERT R. SEALOCK (by invitation), JOHN R. MURLIN and ROBERT L. DRIVER (by invitation). University of Rochester, Rochester, N. Y.

In a previous report from this laboratory, it has been demonstrated that hexyl, heptyl, and octyl resorcinol in strongly buffered alkaline solutions promote the absorption of insulin from the gastro intestinal tract of dogs. This finding warrants the testing of other compounds of a similar nature for this effect. For this purpose the following acute experimental procedure has been adopted. A short length of the upper small intestine of a dog deeply anesthetized with sodium amytal, is cannulated at each end. The compound to be tested is introduced into the intestinal loop in 0.005 molar concentration in 50 cc. of sodium carbonate—bicarbonate buffer at pH 10–10.5, containing 50 units of insulin. At the end of half an hour, the solution is washed out with 0.85 per cent NaCl. Blood samples are removed at intervals and analyzed for glucose by the Shaffer-Hartman-Somogyi procedure. In the case of a positive effect of the insulin solution the blood sugar promptly returns to the normal level after removal of the solution from the loop. By means of this method it is possible to test on one animal the absorption of insulin as influenced by several different compounds.

In extension of the alkyl resorcinol series, it has been found that pentyl resorcinol promotes insulin absorption as do the above compounds, although to a slightly less extent than does hexylresorcinol. The butyl and propyl compounds are significantly less effective than the above members of the series, and the ethylresorcinol has proven completely ineffective as have the acyl resorcinols of this series and the butyl ester of $\beta$-resorcylic acid. The results further indicate, in brief, that the monohydroxy phenols such as tyrosine, p-tert-butylphenol and the butyl esters of the isomeric monohydroxy benzoic acids and the trihydroxy phenols, as, for example, n-butyropyrogallol, do not influence the absorption of the hormone.

*Blood sugar and blood choloride changes during the alarm reaction.* HANS SELYE. Department of Anatomy, Histology and Embryology, McGill University, Montreal, Canada.

We have shown in our previous communications (H. Selye: Endocrinol. **121:** 169, 1937; Nature **138:** 32, 1936; Science **85:** 247, 1937) that exposure to damaging agents elicits a syndrome, the symptoms of which are always the same, irrespective of the agent used. The general resistance increases during this reaction, so that it has been regarded as a defence reaction and

the somatic expression of the "alarm" caused by a stimulus to the quality or intensity of which the organism is not adapted. Until we learn more about the mechanism of this reaction, we shall simply refer to it, therefore, by the non-committal term the "alarm reaction."

After the morphological symptoms of the alarm reaction had been described in the above mentioned publication, it seemed of interest to establish the blood chemical alterations occurring during its course. 200 female rats, two months of age, weighing approximately 100 gms. have been used. They were divided into three experimental groups of 64 animals, while the remaining 8 acted as untreated controls. In the first group, the alarm reaction has been produced by forcing the animals to run 15 minutes, 3 times daily in revolving cages, in the second group, by the subcutaneous administration of 0.2 cc. of a 4 per cent formaldehyde solution thrice daily, and in the third group, by continuous exposure to a temperature of $+1°C$. In each of these three groups, 8 animals were killed by bleeding, 1 hour after beginning the experiment and the same number after 6 and 12 hours, 1, 2, 4, 6, and 21 days respectively. The chloride (Van Slyke) and sugar (Shaffer-Hartmann-Somogyi) content of their blood was determined in each case after 24 hours fasting.

In all groups, the blood sugar increased during the first hours, then a sudden drop to hypoglycemic levels occurred around the 12–24th hour. As adaptation was established a second rise ensued up to a slightly hyperglycemic level. The chlorides showed a sharp decrease which during adaptation was followed by a gradual rise above the normal level. Only in the exercised group did we miss the initial hypochloremia, otherwise these changes occurred irrespective of the stimulus used so that we regard them as signs of the alarm reaction caused by damage as such.

*The renal reabsorption and excretion of urea under conditions of extreme diuresis.* JAMES A. SHANNON. Department of Physiology, New York University College of Medicine, New York City. (Read by title.)

The present report concerns an examination of the renal reabsorption of urea in the dog at very high urine flows. By the intravenous administration of glycose, sulphate and urea we have attained urine flows of 40 to 50 ml. per minute in moderately sized dogs. At these extremely high urine flows the creatinine U/P ratio may be reduced as low as 2.0, and the urea clearance may rise to 90 per cent of the creatinine clearance. The degree of reabsorption of urea at urine flows from 10 to 50 ml. per minute cannot be explained on the hypothesis of passive diffusion in a simple system where the chief determinants of diffusion are the extent of water reabsorption and the time of contact for diffusion as calculated from bladder urine. The inadequacy of the diffusion hypothesis has been previously demonstrated at urine flows from 0.05 to 10.0 ml. per minute.

Our observations lead us to infer that the diuresis occurring under these conditions is not due to the concentration of glucose in the final urine, nor to any osmotic limitation imposed on the reabsorption of water in the thin segment or the distal tubule. They agree better with the thesis that a considerable fraction of the water of the glomerular filtrate is reabsorbed in the proximal tubule without osmotic concentration, that the presence of a high concentration of unabsorbed glucose in the proximal tubular urine limits this reabsorptive process, and consequently, that fluid is delivered from the proximal to the distal portion of the tubule too rapidly for the normal reabsorptive process in this segment to go to completion.

In this view the large deficit in the urea clearance at a creatinine U/P ratio of 10, the lowest obtaintable under water diuresis, is in some manner a consequence of this proximal reabsorption of water.

*The tubular reabsorption of xylose in the dog.* JAMES A. SHANNON. Department of Physiology, New York University College of Medicine, New York City.

The reabsorption of xylose at plasma concentrations of 50 to 150 mgm. per cent is small and shows no tendency to approach a maximal rate when the plasma concentration is raised, in contra-distinction to the reabsorption of glucose, where a maximal rate is demonstrable (Fisher and Shannon). Under the premise that xylose and glucose are actively reabsorbed by a common mechanism, the reabsorption of xylose has been examined at various plasma concentrations of glucose. As the plasma glucose is increased the xylose clearance rises and at levels where frank glycuresis occurs is identical with the creatinine clearance. This identity is maintained as the plasma glucose is elevated to 1500 mgm. per cent. Thus glucose, when presented in excess to the reabsorptive mechanism, completely blocks the reabsorption of xylose, confirming the above premise. There is no evidence of passive diffusion of xylose in the dog's kidney. The quantitative relationship between plasma concentration and reabsorption of xylose are constant with the hypothesis that the quantity of cellular constituent available for combination with glucose and xylose constituents the limiting factor in the reabsorption of both sugars.

*The ratio of arterio-venous differences of certain substances to quantities secreted by the mammary gland.* J. C. SHAW and W. E. PETERSEN (introduced by M. B. Visscher). Department of Dairy Husbandry, University of Minnesota, Minneapolis.

Arterio-venous differences were determined on bloods drawn from the internal iliac and external pudic arteries and the subcutaneous abdominal milk veins for calcium, glucose, lactic acid, fat and amino acids.

The ratio of volume of blood flow traversing the gland to the amount of milk secreted was 1:387 when calculated from the blood calcium differences and the calcium in the milk. The ratio of the combined glucose and lactic acid differences to the lactose in the milk was 1:391 which is in good agreement with the calcium ratio. The differences in neutral fat indicated a ratio of 344 volumes of blood per volume of milk which shows good agreement with the former two when it is postulated that some of the fat is used as a source of energy for the gland by oxidation, which in turn will account for the lower fatty acids found in milk fat. The amount of amino acids removed by the gland are inadequate to account for more than 40 per cent of the proteins of the milk on this basis, as 1087 volumes of blood would be necessary for each unit of volume of milk.

The essential data follow.

| | NUMBER OF ANALYSES | ARTERIAL VENOUS DIFFERENCE | PER CENT IN MILK | RATIO |
|---|---|---|---|---|
| | | *mgm. per cent* | *mgm.* | |
| Amino-acid nitrogen | 15 | 0.46 | 500 (Total nitrogen) | 1–1087 |
| Glucose + lactic acid | 17 | 12.44 | 4860 (Lactose) | 1–391 |
| Fat | 39 | 11.63 | 4000 | 1–344 |
| Calcium | 20 | 0.31 | 120 | 1–387 |

*Investigations on the skin temperatures of the extremities under various controlled environmental conditions.* CHARLES SHEARD, MARVIN M. D. WILLIAMS (by invitation) and BAYARD T. HORTON (by invitation). Division of Biophysical Research and Division of Medicine, The Mayo Foundation and The Mayo Clinic, Rochester, Minn.

This report continues and amplifies our previous investigations concerning the relative rôles of the upper and lower extremities in the control of the exchanges of heat between the environment and the body in normal individuals, in the basal state, under varied but controlled environmental conditions. Data will be presented to show that it is possible to determine the ranges of environmental conditions under which the upper and lower extremities, respectively, commence to function as the regulators of the dissipation of heat from the body.

Determinations have been made of the rates of cooling and warming of various areas of the skin, particularly of the hands and feet, when normal individuals were placed under environmental conditions markedly different from those to which they had been subjected previously. The range of rates of cooling of the toes was found to lie between 0.025 and 0.050°C. per minute. The data obtained concerning the rates of warming of the toes indicate that there are two, and possibly three, distinct rates, ranging from 0.015 to 0.110°C. per minute. Possible explanations for these findings will be given.

The relationships between the basal metabolic rates and the temperatures of the extremities of a number of normal subjects have been investigated under constant environmental conditions (25°C. and 40 per cent relative humidity). The data show that there are two distinct groupings, each of which shows a linear relationship between the temperatures of the toes and the basal heat production. The rôle of the physical and physiological factors affecting these relationships will be discussed.

*The relation of oestrogenic substances to thyroid function and respiratory metabolism.* T. C. SHERWOOD (introduced by W. J. Meek). Department of Anatomy and Physiology, University of Kentucky, Lexington.

The administration of amniotin to ovariectomized rats (group I) produced a decrease of 10–58 per cent in the heat production. All females in the present investigation were approximately 150 days old.

When ovariectomized animals were fed 1000 mgm. of desiccated thyroid per kilo of body weight daily for three days (group II), the heat production was increased approximately 75 per cent. The administration of amniotin, which immediately followed thyroid therapy, returned the animal to the normal level in one-half (four days) the usual time.

No measurable affect was noticed when amniotin was given thyroidectomized animals (group III). The removal of the thyroid glands decreased the heat production approximately 30 per cent.

A group of twelve adult females were ovariectomized and thyroidectomized (group IV). The animals showed an 80 per cent increase in heat production following the administration of desiccated thyroid. Amniotin injections given immediately after thyroid therapy caused a return to the hypothyroid level in approximately seven days which was a considerable reduction from the normal (26 days).

*The respiratory metabolism and lactic acid cycle in the excised cardiac muscle of the depancreatized-hypophysectomized dog.*[1] EPHRAIM SHORR, JOSHUA E. SWEET and MURIEL MALAM (by invitation). Departments of Medicine and Experimental Surgery, The New York Hospital, and the Cornell University Medical College, New York City.

In a previous study from this laboratory (Amer. J. Physiol. **116:** 1936), it was found that excised skeletal muscle from depancreatized-hypophysectomized dogs had partly regained the capacity to utilize carbohydrate, lost when the pancreas alone was removed. The lactic acid oxidative quotient was found normal; and the level of the respiratory quotients, both basal and in the presence of lactate, was intermediate between the values obtained with skeletal muscle from normal and diabetic dogs.

A similar comparison has been made of the carbohydrate metabolism of another tissue, cardiac muscle, from these three types of animals. Data obtained with cardiac tissue from normal and depancreatized dogs has already been reported (Shorr, Malam and Richardson, Amer. J. Physiol. **119:** 1937). Four Houssay dogs, who exhibited no fasting glycosuria, were studied by methods previously described (ibid.). They had been observed in a respiratory calorimeter (Chambers, Sweet, and Chandler, Amer. J. Physiol. **119:** 1937) and found to have respiratory quotients averaging 0.78 under basal conditions, and 0.81 with glucose.

*Results:* In contrast with cardiac tissue from depancreatized dogs, that from the Houssay dog gave respiratory quotients, under basal conditions as well as in the presence of glucose and lactate, which agreed with the normal values. The lactic acid oxidative quotients were also in the normal range. Stimulation of respiration with lactate was somewhat greater than normal. The extent of the carbohydrate metabolism *in vitro* was greater than that exhibited by the whole animal.

*Conclusion:* Excised cardiac muscle from Houssay dogs appears able, independently of insulin, to carry on a normal type of carbohydrate metabolism.

*Comparison of the respiratory metabolism and lactic acid oxidative quotient of excised cardiac muscle from normal, depancreatized, and Houssay dogs*

| TYPE OF ANIMAL | NUMBER OF EXPERIMENTS | RESPIRATORY QUOTIENT | | | OXYGEN CONSUMPTION | | | LACTIC ACID OXIDATIVE QUOTIENT |
|---|---|---|---|---|---|---|---|---|
| | | Non-nutrient | m/80 lactate | 0.2 per cent glucose | Non-nutrient | m/80 lactate | 0.2 per cent glucose | |
| | | | | | *cc. per gram per hr.* | *cc. per gram per hr.* | *cc. per gram per hr.* | |
| Normal | 9 | 0.84 | 0.95 | 0.97 | 0.58 | 0.91 | 0.53 | 3.6 |
| Houssay | 4 | 0.88 | 0.95 | 0.95 | 0.34 | 0.60 | 0.34 | 2.8 |
| Depancreatized | 8 | 0.79 | 0.80 | | 0.86 | 1.15 | | 8.6 |
| Depancreatized | 7 | 0.75 | | 0.73 | 0.63 | | 0.68 | |

*Vasoconstriction of the hand and forearm from a deep inspiration.*[2] ISRAEL SHULMAN (by invitation), MICHAEL G. MULINOS (by invitation) and

[1] Aided by a grant from the Committee on Research in Endocrinology of the National Research Council.

[2] The expenses of this research were defrayed in part by the William R. Warner Fund.

CHARLES C. LIEB. Department of Pharmacology, College of Physicians and Surgeons, Columbia University, New York City.

A study of the vascular reaction of the hand and forearm to a deep breath was made by the use of 5 distinct methods. (1) The pressure plethsmograph method of Hewlett (Heart **1:** 87, 1909), measures total flow into the hand and forearm; while (2) the plethysmograph shows changes in volume. (3) The insulated thermocouple measures skin temperature, and (4) the conducting thermocouple shows skin blood flow in terms of temperature changes. (5) Microscopic observation of the capillaries shows the size, shape and number of these vessels, as well as the speed of blood flow through them. Each experimental subject was tested by all 5 methods.

A deep inspiration caused vascular constriction in the forearm as measured by the foregoing 5 methods, in over 300 experiments upon 63 subjects, including 8 with thrombo-angiitis obliterans and 10 with Raynaud's disease. The constriction is a reflex initiated by the inspiratory movement, as shown by its independence of blood flow (Proc. Soc. Exper. Biol. and Med. **34:** 89, 1936) and its dependence upon intact nerves (J. Physiol. **86:** 83, 1936). The reflex is not due to cooling, for it persists if the inspired air is warmed and moistened. The pressure plethysmograph shows complete stoppage of flow in 60 per cent of all subjects, confirmed by direct observation of flow in the capillaries. The skin temperature from a single deep inspiration shows no change, while the conduction thermocouple registers a drop of from 0.1° to 1.0°C. Ten deep breaths at 10 second intervals caused no additive change in blood inflow (the Hewlett plethysmograph); a shrinkage of the forearm only slightly greater than from one breath; and a drop in skin temperature of 0.24° to 1.7°C., a distinctly additive phenomenon. These results show that a deep breath of air caused a constriction of the arterioles of the forearm; and that there is a greater and more lasting effect upon the vessels of the skin than upon those of the deeper structures.

*Phenomena allied to conditioning in the spinal dog.* P. S. SHURRAGER (by invitation) and E. A. CULLER. University of Illinois, Urbana.

In young dogs, under complete anesthesia, the cord is transected at the third cervical root; mechanical respiration is maintained; both semitendinosus muscles (S–T MM.) are freed of fascial attachments, with the nerve- and blood-supply intact; the muscles are detached at both their insertions and their ischiatic origins; the hind-quarters are held rigidly by a drill rod through the femurs and inverted in a "U"-shaped support, where the origins are rigidly fixed; and the insertion of each muscle is attached to a kymograph lever. Simultaneous contralateral records are made.

The "conditioning stimulus" (CS) may be either mechanical or electric, and is applied to the tip of the tail. Moderate pressure with a stiff brush, an A.C. potential of one volt across the tail (30,000 ohms) or a D.C. pulse of 100 micro-amperes is generally sufficient.

The "unconditioned stimulus" (UCS) is an A.C. or D.C. shock of adequate intensity to elicit a full contraction of the left S–T M. when applied to the left paw.

The technique generally used employs two CSs with one second interval, followed one second later by both CS and UCS simultaneously. The "conditioned response" (CR) to the CS alone generally appears within

25 to 50 trials at intervals of thirty seconds. The CR is much smaller than the response to the paw-shock (UCR) and is generally confined to a small area near the peripheral end of the muscle. The right muscle remains flaccid throughout.

Repeating single or double CSs (without UCS) at intervals of thirty seconds "extinguishes" the CR. Repeated alternations of "conditioning" and "extinction" indicate that both occur more readily after repetition.

Relatively intense stimulation of the tip of the tail may call forth a spinal reflex of the right S–T M., the left remaining flaccid. By use of this intense stimulation as a CS, the left S–T M. can be conditioned. With the gradual increase of the CR in the left S–T M. the reflexive response in the right S–T M. has been observed to diminish and finally disappear.

The reflexive response of the right S–T M. to the intense CS is larger and has a longer latent period than does the CR in the left muscle from the same CS.

*Phenomena allied to conditioning in the spinal dog.* P. S. SHURRAGER (by invitation) and E. A. CULLER. University of Illinois. (Motion picture.)

This film will illustrate some of the phenomena described in the preceding abstract.

*The action of benzyl methyl carbinamine sulfate, S. K. F., on the coronary blood flow of dogs.* K. A. SILER (introduced by O. O. Stoland). Department of Physiology, University of Kansas, Lawrence.

Studies on the effect of adrenaline (epinephrine) and ephedrine on the coronary blood flow of dogs in this laboratory (Stoland and Ginsberg) have shown that these vaso-constrictors cause an increased flow of blood through the coronary vessels of the dog's heart. The recent introduction of a synthetic vaso-constrictor which is being used clinically in many different conditions prompted an investigation of its action on the coronary circulation. Flow per minute was determined by collection of blood from the coronary sinus by means of the Morawitz cannula in denervated heart-lung preparations and the intact animal. Following small intravenous doses (0.01–0.1 mgm.) there is a simultaneous rise in blood pressure and coronary flow in the denervated heart-lung preparation.

In one dog a larger dose (1 mgm./kg. body weight) which was apparently toxic caused a marked transient rise in blood pressure after which the pressure remained about 10 per cent above the original level. Periods of cardiac irregularity occurred. The coronary outflow in this dog was increased by about 400 per cent.

The subcutaneous injection of 10 mgm. of this drug into the intact animal causes a gradual, sustained increase in both blood pressure and coronary outflow.

The rise in coronary flow which follows the administration of small non-toxic doses of benzyl methyl carbinamine sulfate can be accounted for, in all our animals, by the coincident rise in blood pressure.

*Interrelationships between potassium and carbohydrates.* HERBERT SILVETTE.[1] Physiological Laboratory, University of Virginia, University. (Read by title.)

[1] E. R. Squibb Fellow in Physiology.

The injection of non-toxic amounts of potassium salts into rats resulted in hyperglycemia over a four-hour period. At the end of this time liver and muscle tissues analyzed for glycogen content showed subnormal glycogen concentrations. Potassium injected into rats, cats and opossums, simultaneously with 5 per cent glucose solution, was followed by glycogenolysis and hyperglycemia. For the same amount of potassium per kilo body weight, the hyperglycemia was greatest and most prolonged in the opossum, and least in the rat. No toxic symptoms were observed to accompany the changes in carbohydrate levels.

*Renal secretion in the opossum.* HERBERT SILVETTE.[1] Physiological Laboratory, University of Virginia, University. (Read by title.)

Female opossums were fasted for 18 hours, then given 2 per cent of various fluids per kilo body weight every 2 hours. After a preliminary period of 6 hours during which the animals came into equilibrium under the experimental conditions, all urine was collected by catheter for three consecutive two-hour periods, measured and analyzed for chlorides and urea. Both normal and adrenalectomized animals were employed. They were given in different experiments distilled water, 0.9 per cent NaCl, or a solution containing 0.9 per cent NaCl and 2 per cent urea by mouth, and the effects of cortico-adrenal extract, post-pituitary solution (Squibb) and adrenalin on the excretion of water, chlorides and urea were determined.

It was found that the adrenalectomized opossums were able to concentrate urea and thus excrete this substance in approximately normal amounts even though the excretion of water and salt was partially inhibited. Even large amounts of ingested urea were easily and rapidly excreted by adrenalectomized animals, in contradistinction to the slow and incomplete elimination of excess sodium chloride. The administration of cortico-adrenal extract was accompanied under all experimental conditions by a decrease in urea concentration per cc. of urine, although the concomitant increase in urine volume resulted in an absolute (total) increase in urea output.

In general it may be stated that any variation in experimental conditions which affects urine volume brings about a change in chloride excretion in the same direction. The kidney of the adrenalectomized animal appears to be unable to concentrate chlorides with the same facility that it concentrates urea.

| FLUID BY MOUTH | NORMAL OPOSSUMS | | | ADRENALECTOMIZED OPOSSUMS | | |
|---|---|---|---|---|---|---|
| | Substance added | | | Substance added | | |
| | Cortico-adrenal extract | Post-pituitary | Adrenalin | Cortico-adrenal extract | Post-pituitary | Adrenalin |
| Water: | | | | | | |
| Water excretion | + | − | + | + | − | − |
| Chloride excretion | + | + | 0 | + | + | − |
| 0.9 per cent NaCl: | | | | | | |
| Water excretion | + | + | + | + | + | − |
| Chloride excretion | + | + | 0 | + | + | − |

[1] E. R. Squibb and Sons Fellow in Physiology.

The table on page 188 shows concisely the effect of various experimental procedures on water and chloride elimination in the opossum. An increased excretion is represented by (+), a decreased excretion by (−), and no change from the control untreated animals by (0).

A noteworthy observation was the marked effect on renal secretion of cortico-adrenal extract given by mouth.

*The effect of sympathetic denervation on the responses of the submaxillary gland of the cat to adrenalin, acetylcholine and pilocarpine.* F. A. SIMEONE and J. MAES (introduced by W. B. Cannon). Harvard Medical School, Boston, Mass.

Experiments were performed comparing the secretion of the normal submaxillary gland with that of the contralateral gland denervated 47 to 90 days previously by excision of the corresponding superior cervical ganglion. Ten cats were used, under urethane anesthesia. In 5, the right gland was denervated; in 5 others the left. Secretion was excited by injections of adrenalin (5 to 500 $\gamma$), acetylcholine (0.25 to 1.5 mgm.), and pilocarpine (0.1 to 0.3 mgm.). The number of drops of saliva from the cannulated ducts were recorded on a kymograph. The chorda tympani was cut acutely on both sides. The adrenal glands were removed.

In 5 control animals, the responses to adrenalin of right and left normal glands were identical except for one case in which one gland was more responsive than the other. In 6 of the 10 experimental animals, the responses of the denervated gland were 2 to 9 times those of the control. In 2 other cats, the secretion was also increased, but less strikingly. In the remaining 2 animals, the responses of the normal and denervated glands were equal.

The responses of the denervated gland to acetylcholine were definitely greater than those of the normal gland in only 3 of the 10 animals. In 4 others, the denervated glands showed only slightly greater responses, and in the remaining 3, secretion on the two sides was equal. Six out of 7 animals tested showed marked sensitization of the denervated gland to pilocarpine. The other showed no sensitization to any of the 3 drugs used.

It is concluded that sympathetic denervation increases the responsiveness of the submaxillary gland to adrenalin and to pilocarpine. Sensitization to acetylcholine is less frequent.

*Observations on the effective renal blood flow and functional excretory mass in man, with special reference to essential hypertension.* HOMER W. SMITH, WILLIAM GOLDRING (by invitation), HERBERT CHASIS (by invitation) and HILMERT A. RANGES (by invitation). Departments of Medicine and Physiology, New York University College of Medicine, New York City.

Methods for measuring the renal blood flow and excretory mass of the human kidney have been applied to normal and hypertensive subjects under basal conditions and during various therapeutic procedures. The hypertensive subject invariably shows a renal blood flow less than normal values, and a filtration fraction greater than is observed either in the normal subject or in subjects with chronic glomerulonephritis of the same degree of renal impairment.

Hypertensive subjects in whom the renal mass is not perceptibly reduced, although showing absolute renal ischemia, have a filtration rate

and urea clearance which are normal, probably in part because of increased tonus of the efferent glomerular arterioles and in part because of increased systemic arterial pressure.

In hypertensive subjects in whom the excretory renal mass is markedly reduced the renal blood flow and filtration rate, although reduced absolutely, may be relatively increased when referred to the mass of the residual normal tissue. Though the systemic hypertension may be contributory to this phenomenon, it must be recognized that blood which once had been cleared by defunct tubules is available, at least within a certain radius of diffusion, to adjacent normal tubules, creating in effect an actual hyperemia of the latter. And insofar as tubules which have lost their excretory capacity retain connection with their glomeruli they may serve as passive conduits to drain glomerular filtrate into the urine, the net result being an excessive filtration rate relative to the mass of residual normal tubules.

Our observations may be interpreted as in accordance with those of Goldblatt and his coworkers and of others who have shown that renal ischemia can induce hypertension in dogs, but the conclusion that renal ischemia is a causal factor in hypertension in man cannot be reached until it is demonstrated that the observed renal ischemia in man is not simply a concomitant of generalized systemic hypertonus.

*Changes in placental transmission at various stages of pregnancy.* FRANKLIN F. SNYDER and HAROLD SPEERT (by invitation). Johns Hopkins University, Baltimore, Md.

The observations represent a quantitative determination of the activity of the placenta at various stages of pregnancy, using as a measure of placental function the amount of arsenic which is found in the fetus after injection of the mother with neoarsphenamine. Under conditions standardized with respect to dosage and time interval following injection, there is revealed a progressive increase in the rate of placental transmission of arsenic from mother to fetus as pregnancy approaches termination.

In a series of rabbit fetuses examined one hour after injection of the maternal animal, no arsenic was detected at twenty-five days of gestation, and at twenty-seven days there was only a small amount (1/4 micromilligram). From the beginning of the period of viability, twenty-eight days, increasingly larger amounts of arsenic were found in the fetus as pregnancy progressed, eight micromilligrams being recovered at thirty-four days, i.e., two days past term.

In a second series of fetuses removed twenty-four hours after injection, arsenic (1/4 micromilligram) was detected at nineteen days. At thirty-four days the arsenic content of the fetus reached twenty micromilligrams.

The curve of arsenic concentration of the fetus was roughly parallel to that representing the total arsenic content.

The placenta contained much more arsenic than the fetus; the average arsenic content of the placenta was 18 times, and the arsenic concentration 175 times, as great as that of a fetus at full-term one hour following injection. Variations in the arsenic content of the placentae could not be correlated with the stage of pregnancy. Gradual liberation of arsenic from the placenta to the fetus is indicated by the consistent finding of a greater amount of arsenic in the fetus twenty-four hours after injection than after one hour.

Analyses are based upon 44 fetuses and 24 placentae obtained from 24 rabbits at various stages of pregnancy from 19 to 35 days. Dosage of neoarsphenamine was 0.020 gram per kilogram body weight administered intravenously in 2 cc. A modified Gutzeit method detected $\frac{1}{2}$ microgram of arsenic trioxide. Readings are expressed in terms of arsenic trioxide.

*Measurement of human nerve accommodation with linearly rising currents.* D. Y. Solandt. Department of Physiology, University of Toronto, Canada.

Keith Lucas has shown that, when using linearly rising currents to excite a nerve, the slower the current rises the higher is the threshold. A. V. Hill more recently has supplied a theoretical treatment of these experiments and demonstrated that Lucas was really obtaining a measure of $\lambda$, the time constant of accommodation. Solandt has described a method of measuring $\lambda$ in human nerve, using exponentially rising currents. The technique using linearly rising currents is similar but simpler. It requires only two threshold determinations.

The linearly rising currents are provided by charging a condenser through a constant-current pentode vacuum tube. Voltage rise across the condenser is then converted into current rise in the output circuit of a single-stage vacuum tube amplifier. The current rise is cut off at the desired level by a thyratron.

Determinations of $\lambda$ were made by exciting the ulnar nerve where it is subcutaneous at the elbow. Muscle twitch was used as index of excitation for motor fibres and a definitely limited sensation used as index for sensory fibres. Instead of employing a single impulse for excitation, the familiar saw-tooth wave used for the sweep circuit of cathode ray tubes may be amplified and used for measuring $\lambda$. In this case index of excitation is tetanus instead of a twitch, or prolonged instead of transitory sensation.

*Physiological factors in effort syndrome.* Mayo H. Soley and Nathan W. Shock (introduced by C. D. Leake). Departments of Medicine and Physiology, University of California Medical School, San Francisco. (Read by title.)

We examined seven patients suffering from effort syndrome by estimating at basal levels the oxygen consumption, concentration of $CO_2$ and $O_2$ in the expired air, $pCO_2$ and pH of finger blood, serum bicarbonate (finger blood) and the ventilation volume per minute and then followed these various factors during $3\frac{1}{2}$ to 10 minutes of forced overbreathing. The patients developed the symptoms of dizziness, fatigue, palpitation, constriction in the throat and chest, sense of suffocation, numbness of the hands and feet and finally tetany when the pH had shifted from the normals of 7.32–7.38 to 7.43–7.55. Thus the level of the pH at which these patients developed tetany was lower than the usual 7.55–7.60 in normal subjects. The shift in pH which we have demonstrated occurred after $2\frac{1}{2}$ to 5 minutes of hyperventilation at 3–8 times the basal respiratory volume. The time required to produce this shift in the pH was inversely proportional to the degree of hyperventilation as measured by the respiratory volume. The oxygen consumption during forced overbreathing increased to 100–180 per cent above the basal level. The $pCO_2$ decreased from 40–44 mm. Hg to around 30 mm. Hg and the serum bicar-

bonate decreased slightly but constantly in all the patients. When $CO_2$ was administered, the ventilation volume was twice that during forced overbreathing of room air but none of the same symptoms appeared. Under $CO_2$ the pH fell to as low as 7.18 and the $pCO_2$ rose to around 65 mm. Hg.

*On the mode of action of insulin.* SAMUEL SOSKIN and R. LEVINE (by invitation). Metabolic Laboratory of the Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago, Ill.

The supposed indispensability of insulin to the oxidation of carbohydrate is largely based upon determinations of the respiratory quotient. Aside from the disputed validity of this criterion, there have been many contradictory results. Using more direct methods (Am. J. Physiol. **120**: 761, 1937), we have recently shown that the rate of carbohydrate utilization depends upon the height of the blood sugar level, whether insulin is present or not. The completely depancreatized dog at his characteristic blood sugar level utilizes as much sugar as the normal dog at the normal level. Above certain high levels even this difference disappears, and both animals utilize the same large amounts of carbohydrate.

We wish to report further work with the above methods. We have found that the administration of large doses of insulin to normal dogs does not significantly affect their rate of sugar utilization at any given blood sugar level. It follows that an optimal amount of insulin is already present in the normal animal. Any amount of insulin administered to the depancreatized animal, can only increase its sugar utilization up to but not beyond that rate which occurs at the same blood sugar level in the normal animal.

This influence of insulin on the relative rate of sugar utilization is similar to its effect upon muscle glycogen deposition. Major and Mann found that muscle glycogen is deposited in the completely depancreatized dog, although at a slower rate than in the normal animal. Moreover, both Dambrosi and Lukens have shown that the restoration of muscle glycogen after muscular exercise is just as complete in the depancreatized as in the normal animal. The difference lies in the time required for the completion of the process.

It is evident that insulin acts by increasing the rate of sugar utilization and of glycogen storage, but that these phenomena do proceed at slower rates in the absence of insulin. Since insulin is not essential to these processes, it is probably not directly involved in them. It is suggested that its action resembles that of an activator or co-enzyme, affecting some intermediate step in carbohydrate metabolism which precedes utilization or storage.

*Conditioned reactions to angular acceleration.*[1] E. SPIEGEL and M. OPPENHEIMER (by invitation). Department of Physiology and the Department of Experimental Neurology, D. J. McCarthy Foundation, Temple University School of Medicine, Philadelphia, Pa.

Conditioned reflexes at certain positions in space were demonstrated in former experiments as a part of systematic studies of the relationship

[1] Aided by a grant of the National Research Council to E. S.

of the labyrinth to higher parts of the central nervous system. Since these reactions are partly of labyrinthine and partly of extralabyrinthine origin, in this series of experiments it was attempted to condition stimuli that act as exclusively as possible upon the labyrinth, namely, angular acceleration. Dogs are first rotated with a slow constant speed by connecting a motor with the apparatus by a cord conducted over pulleys, forming a loop like an inverted U. After 2 to 10 rotations a weight is attached by a clamp to the highest part of the descending limb of the loop, so that the constant speed passes smoothly into accelerated rotation. During the latter, the unconditioned stimulus, (electric shock) is applied to a leg. Optic reflexes are excluded by a hood; stimulation of the body surface by air currents arising during rotation is avoided by covering the body. A few (8–15) repetitions of combined application of the conditioned and of the unconditioned stimulus usually are sufficient, so that after omitting the latter stimulus the accelerated rotation alone elicits withdrawal of the leg which was absent before the training. The reaction shows typical qualities of conditioned reflexes, e.g., anticipatory reactions, extinction. The smallest acceleration with which a conditioned reaction could be produced was 2°–3°/sec.$^2$ Yet the threshold may be still lower, since smaller accelerations could not be produced by the apparatus without causing irregularities of the motion. Discrimination of direction was developed if only one direction was associated with the unconditioned stimulus. After elimination of the labyrinths conditioned reactions could no longer be produced at low acceleration, and discrimination of direction was lost at low and high acceleration; combined section of the posterior and dorsal spinocerebellar tracts did not prevent the development of these reactions.

*A consideration of androgens as corticosterone substitutes.* CHARLES L. SPURR and CHARLES D. KOCHAKIAN (introduced by John R. Murlin). University of Rochester, Rochester, N. Y.

The closely related embryological origin of the adrenal cortex and the testes and the similarity in the chemical nature of the hormones elaborated by these 2 organs, suggest a possible mutual physiological action by these substances. Since pure androgens are readily available and the adrenal cortical hormone (corticosterone) is scarce, a study of the use of androgens as a substitute for corticosterone became desirable.

In order to make the test as crucial as possible, the survival time of adrenalectomized male white rats weighing 40–60 grams was used. Observations were made on groups of ten or more animals after careful total extirpation of the glands under nembutal anesthesia. The day after the operation the animals were injected subcutaneously until death with the prescribed androgen dissolved in sesame oil so that the required daily dose was administered in 2 doses (A.M. and P.M.) of 0.05 cc. each. Controls were treated in an identical manner with sesame oil.

The daily administration of the synthetic androgens: androstenedione (0.5 mgm. 4.2 I.U.; 1 mgm. 8.4 I.U.; 3 mgm. 25 I.U.) testosterone acetate (0.28 mgm., 14 I.U.) and testosterone propionate (1 mgm. 50 I.U.) did not significantly increase or decrease the average survival time of the rats from that of the controls of 7.1 days. On the other hand, an extract of human male urine decreased the average survival as the dose was increased. Thus with 38 I.U./day the survival period was 6.2 days; 116

I.U./day, 4.5 days and 194 I.U./day only 2.7 days. The causative agent in this shortening of life is not known.

*Changes in the total blood volume in histamine and surgical shock in normal rabbits and in those pretreated with histamine.* S. KARÁDY (introduced by J. C. Meakins). McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

It has previously been shown by Rusznyák Karády and Szabo (1937) that it is possible to prevent surgical shock in human beings by pretreatment with histamine. In the present investigations the blood volume was determined by the method of Gibson and Evans, using the Evans Blue dye and the Evelyn photo-electric micro-colorimeter. It was possible to estimate blood volume using only 0.5 cc. for each sample. The changes in haemoglobin concentration were also followed on the photo-electric colorimeter. The animals were anaesthetized with urethane and chloralose, the blood volume was determined, then either histamine or surgical shock was produced, the former by the subcutaneous injection of 5 mgm. of histamine hydrochloride per kg. body weight, the latter by opening the abdomen and handling the intestines for a period of five minutes. The blood volume was re-determined one hour after these procedures. The pretreated group was given subcutaneous injections of histamine for from 7 to 10 days in gradually increasing doses (5, 10, 15, 20 mgm. continuing at 20 mgm. thereafter daily). The procedure in the case of these animals was the same as for the control group and the blood volume was determined before, and one hour after histamine or surgical shock.

The results were as follows: the non-pretreated group showed a decrease in blood volume of from 24–53 per cent after histamine injection and a decrease after surgical shock of from 13–56 per cent. The pretreated group on the other hand showed after the same time period an increase in blood volume of from 5.2–23 per cent after histamine and of from 6–28 per cent after surgical shock. The haemoglobin concentration and haematocrit reading showed changes which were in agreement with the changes in blood volume. In one animal in histamine shock the concentration of the dye was determined in the peripheral, pulmonary and portal venous blood simultaneously. The concentration was the same in all three samples.

Their results are in accordance with the experiments of Rusznyák Karády and Szabo using the congo red method in the dog.

*The metabolism of Trichinella spiralis larvae.* J. N. STANNARD and O. R. MCCOY (introduced by W. O. Fenn). Department of Physiology and Laboratory of Parasitology, Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

The object of this work was a study of the general characteristics of the metabolism of a nematode parasite of muscle tissue, especially in comparison with the metabolism of the host muscle tissue.

The metabolism of mature larvae of *Trichinella spiralis* (digested free from infected rat muscle was studied by standard microrespirometer techniques. The rate of oxygen consumption in Tyrode's medium was fairly high ($-Q_{O_2} = 2.24 \pm 0.31$ in 53 experiments) and considerably higher than the rate of anaerobic $CO_2$ production ($Q_{CO_2}^{N_2} = 1.17 \pm 0.22$ in 10 experiments). The respiratory quotient, determined by two methods,

was slightly above one. It is believed that this indicates a residue of fermentation proceeding under aerobic conditions.

The addition of glucose, glycogen, lactate, pyruvate, succinate, hexose diphosphate, serum, or Douglas' medium did not cause any significant rise in $-Q_{O_2}$. No glucose was removed from Tyrode's medium even after four days under either aerobic or anaerobic conditions. Under the conditions of the experiments, it appeared that the larvae were utilizing endogenous substrate.

The rate of respiration was independent of oxygen tension above one per cent. Oxygen consumption was inhibited markedly by cyanide, and stimulated by paraphenylene diamine and carbon monoxide. This latter is characteristic of muscle tissue only. Hemoglobin was present in the larvae in low concentration.

The fermentation unlike the glycolysis of muscle consisted in the production of gaseous carbon dioxide and other products which cannot be acidic in nature, because the rate of carbon dioxide production was identical in phosphate or bicarbonate buffers, and in pure nitrogen or mixtures of $CO_2$ and nitrogen. This fermentation was only slowly inhibited by 0.00054 M iodoacetate or iodoacetamide and 0.055 M to 0.10 M fluoride; concentrations known to inhibit rapidly the glycolysis of muscle. On the other hand the respiration was as quickly inhibited by these substances as the respiration of muscle. Arsenite inhibited respiration more quickly than fermentation.

The respiration is rather similar in these general characteristics to that of muscle while the anaerobic metabolism is relatively dissimilar. No evidence was found of a Pasteur-Meyerhof reaction in the larvae.

*Apparatus for recording the heart's recoil and the blood's impacts in man, (ballistocardiograph), experiments on the principles involved, records in normal and abnormal conditions.* ISAAC STARR, A. J. RAWSON (by invitation) and H. A. SCHROEDER (by invitation). Univ. of Pennsylvania, Philadelphia.

Our apparatus is a modification of that of Y. Henderson (1905). A rigid bed is hung from the ceiling on wires and braced to prevent motion in any but the longitudinal direction. Motion in this direction, opposed by a strong spring, is magnified 500 times and photographed. Subjects lie on this bed.

With steel weights equivalent to body weight on the bed the vibration period may be varied by altering the spring tension. The highest period conveniently attained is about 20/sec for light subjects. When the steel weights are replaced by a cadaver of the same weight the period is greatly changed becoming about 6/sec and this can be influenced little if at all by adjusting the tension of the spring. Therefore the body's period dominates the apparatus. The body also provides damping of a high degree. These results raise the question whether anything is gained by the use of the elaborate and expensive apparatus necessary to secure a very high natural period (Abramsen 1933) when the apparatus is weighted by iron.

The normal record shows a headward movement during the preejection period, a sharp feetward impact at the beginning of ejection, obviously cardiac recoil; a headward impact shortly thereafter, blood striking the aorta; a final feetward impact, blood striking the lower parts of the body.

The amplitude is related to cardiac output. It is increased by food, exercise, excitement and by drugs which stimulate the circulation.

In certain pathological conditions the form of the record is profoundly altered.

*Phosphocreatine changes in normal and leaded muscle.* S. E. STEIMAN (introduced by H. Davis). Department of Physiology, Harvard Medical School, Boston, Mass.

Frogs were poisoned with lead by keeping them in an approximately 0.01 per cent $PbCl_2$ solution. Experiments were made on the animals after varying intervals of exposure. A day before the experiment the animal was placed in a cold room (3–8°C.), where all further procedures were carried out. Under nembutal anesthesia, the sciatic of one leg was exposed and stimulated with a tetanizing current for 2 minutes. The opposite unstimulated gastrocnemius served as a control. After varying periods of recovery the stimulated and control muscles were frozen in liquid air and analyzed for phosphocreatine by the Fiske and Subbarow method. A series of experiments on normal animals was also done.

Phosphocreatine is expressed here as milligrams per cent of P. The average value for normal unstimulated muscle was 56.2 $\pm$.85, while at the end of 2 minutes' stimulation it was 27.2 $\pm$1.9. The resynthesis of phosphocreatine is rapid during the first minute of recovery and from then on is much slower. At the end of 30 minutes the phosphocreatine is restored to the resting level.

In 24 experiments the resting level of phosphocreatine in muscles of leaded animals (2 to 20 days) was lower (37.1 $\pm$1.6), and after 30 minutes' recovery from stimulation it was not completely resynthesized (29.9 $\pm$1.4). It was further found that exercise produced even greater changes with short periods (2 to 5 days) of exposure to lead. The resting level of phosphocreatine was still lower (24.2 $\pm$2.9), while after 30 minutes' recovery from stimulation it was 15.3 $\pm$1.5. The figures obtained from leaded non-exercised animals for the same time of exposure were 37.6 $\pm$2.9 for the resting level of phosphocreatine and 32.0 $\pm$2.7 after 30 minutes' recovery.

These results show that lead causes a fall in the resting level of phosphocreatine and also prevents or retards its resynthesis. Since phosphocreatine is necessary for contraction, the impairment of its metabolism by lead is probably an important factor in causing muscular paralysis in lead poisoning.

*Evidence of a secretory mechanism in the chorioid plexus.*[1] ROBERT D. STIEHLER (by invitation) and LOUIS B. FLEXNER. Departments of Ophthalmology and Anatomy, Johns Hopkins Medical School, Baltimore, Md.

Observations indicate that in the chorioid plexus an electric current between stroma and epithelium causes cations to move from stroma to epithelium and anions in the opposite direction. The energy for this current is supplied by oxidative and reductive processes. The evidence for these views is as follows: (1) At physiologic pH, selected basic dyes go

[1] This investigation was supported in part by a grant from the John and Mary Markle Foundation.

rapidly from stroma to epithelium but not in the reverse direction. With asphyxia, basic dyes pass in both directions but with difficulty. At physiologic pH, selected acid dyes pass rapidly from epithelium to stroma but not in the reverse direction. With asphyxia, acid dyes pass readily in both directions. (2) There is a basement membrane between epithelium and stroma which can be studied under asphyxia. Basic dyes pass this membrane with difficulty on the alkaline side of the isoelectric point and easily on the acid side. The opposite is true for acid dyes. (3) Indophenol-oxidase is present in high concentration in the epithelium but is absent in the stroma. Correlated with this, the measured aerobic oxidation-reduction potential of the epithelium is approx. 100 millivolts more positive than that of the stroma.

*Phases in the assimilation of carbohydrate and fat by bakers' yeast.* THEODORE J. B. STIER and HERBERT SPRINCE (introduced by Alfred C. Redfield). Biological Laboratories, Harvard University, Cambridge, Mass.

Previous investigations on the assimilation of carbohydrate and fat by various yeasts have been concerned mainly with the yields of the anabolic products formed after some arbitrary time interval. In this paper we shall describe preliminary results from a research program utilizing the kinetic approach in investigating the assimilation mechanism of intact yeast cells.

Fresh bakers' yeast (Fleishmann GM strain) was brought to a minimum level of carbohydrate and fat content by aerating for 3 days at 25°C. in M/15 $KH_2PO_4$. This yeast was then centrifuged and resuspended in M/15 $KH_2PO_4$—15 per cent. glucose solution at 5°C. Samples were withdrawn and fixed at various time intervals for determinations of dry weight, total carbohydrate, glycogen, and total fat.

Under aerobic conditions the observed phases are: (1) an induction phase lasting from 0 to 6 hr. (practically no change in dry weight or in total carbohydrate); (2) a period of active carbohydrate assimilation, from 8 to 27 hr. (no further increase in total carbohydrate after 27 hr.); and (3) a period of further increase in dry weight which we attribute to the formation of fat. The dependence of fat formation on the presence of assimilated carbohydrate and the alcohol formed during assimilation is now being investigated.

Under anaerobic conditions the induction phase also lasts about 6 hr.; thereafter, the total carbohydrate content increases until about 72 hr. and then remains constant with time. After 72 hr. the dry weight content increases very slowly. Fat analyses have not been completed as yet. A "Pasteur effect" on the assimilation process is observed.

Evidence will be presented in support of the view that the synthesis and storage of "reserve food materials" is a basic "physiological activity" of intact yeast cells metabolizing glucose (or a comparable substrate) and that results from experimental work on the metabolism of such cells should be evaluated with reference to the phases of this fundamental process.

*Hemolysis resulting from hyperventilation.* R. T. STORMONT (by invitation), F. E. SHIDEMAN (by invitation), T. J. BECKER (by invitation), and M. H. SEEVERS. Department of Pharmacology, University of Wisconsin, Madison.

Incidental to other studies on barbitalized dogs during prolonged hyperventilation, it was noted that hemoglobinemia and hemoglobinuria occur-

red. In a series of animals studied subsequently, blood samples were taken at intervals, heparinized, centrifuged, and examined for hemolysis. It was found that hemoglobinemia could invariably be produced if hyperventilation was sufficiently severe and prolonged. Hemolysis was noted, without exception, in twenty-four dogs subjected to hyperventilation, in which the average arterial blood $CO_2$ content decreased from 45 to 15.5 volumes per cent, the average pH value for arterial plasma increased from 7.42 to 7.74, and an average rise in blood lactic acid from 13 to 58 mgm. per cent occurred. The interval between the onset of hyperventilation and the appearance of hemolysis varied from 33 minutes to 4 hours. Eight of nine dogs, in which recovery was attempted, survived even though severe hemoglobinemia was present.

Attempts to determine the factor or factors causing the hemolysis have thus far been without complete success. No significant change in the depression of the freezing point of blood plasma occurred, and changes in the chloride concentrations in plasma and cells were slight and inconstant. Although blood concentration occurred often, as judged by hematocrit determinations, it was not an invariable finding. No alteration of the fragility of corpuscles was observed until just prior to the onset of hemolysis. The appearance of hemolysis was not prevented by ventilation with heated moist air or with oxygen. The administration of ascorbic acid prior to and during hyperventilation did not prevent hemolysis.

If the normal level of arterial blood $CO_2$ was maintained by ventilation with an air-$CO_2$ mixture, no hemolysis occurred even though hyperventilation of the same mechanical severity as in the above experiments was continued for 9 hours.

Factors such as the prolonged exposure of the cells to the high pH of the plasma, or some specific effect resulting from the loss of $CO_2$, are being investigated as possible causes of the phenomenon.

*A histochemical index of rates of neural oxidations.* O. SUGAR (by invitation) and R. W. GERARD. Department of Physiology, University of Chicago, Ill.

Analysis of the relative respiration rates of small brain regions, carried ultimately to parts of the neurone, as cell body, dendrites, synapses, is most desirable; for gross averages on a tissue mass containing many cell types (including glia) are almost uninterpretable. If it could be definitely shown, for example, that synapses do or do not have a higher metabolism it would favor respectively the humoral or the electrical theory of synaptic transmission. Though capillary lengths shows consistent quantitative variation from one histologic region to another, quantitative inferences as to respiratory rates are unsound (Gerard, Trans. Ass. Res. Nerv. & Ment. Dis., 1938).

An oxidizing agent substituted for oxygen should initially be reduced in proportion to the normal oxidative rate. If this agent also kills rapidly, and if its reduction product can be determined in sections, a histologic picture of the original respiration intensities should result. Ferric chloride kills and fixes rapidly, but not before significant reduction has occurred. The resulting ferrous salt is readily identified as the blue ferricyanide; the remaining ferric salt as the ferrocyanide. Rapidly frozen brain or spinal cord can be sectioned, and the slices thawed and passed through ferric chloride, Ringer, and ferricyanide. Perikarya, dendrites, and axones stain

blue in order with increasing concentration of ferric chloride or time of immersion in it.

Ferric chloride can be reduced by tissue substances aside from the normal respiration catalysts, for slices of killed (formol, alcohol, dichromate) brain are also stained blue, at once or more slowly. Cell outlines may be distinguished but staining is diffuse and fairly homogeneous in such slices or others killed in ferric chloride while still frozen (respiration suspended), or slowly frozen and thawed repeatedly. The characteristic differential reduction of ferric chloride by neurones depends, therefore, on their being alive and respiring.

Results will be shown with microphotographs. As an example, the cell layer of the rat hippocampus is well stained, while the immediately underlying neuropil (richly synaptic) is not. If poor reduction by neuropil is consistent it indicates low synaptic metabolism and hence is unfavorable to the humoral theory of synaptic transmission.

*Temperature effects on serum volume.* F. W. SUNDERMAN (by invitation), J. C. SCOTT and H. C. BAZETT. Departments of Physiology, Medical School, University of Pennsylvania, and Hahnemann Medical College, Philadelphia.

Using the same subjects repeatedly, seasonal variations in the serum and blood volumes have been detected. For the measurement of serum volume a modification of the Sunderman and Austin Method was employed. Blood volume was calculated from the measurements of serum volume and hematocrits. In three subjects examined during cold weather and subsequently one or more times in hot weather, the blood volume in hot weather increased from 15 to 40 per cent above values obtained in cold weather.

Observations were made on subjects A and B maintained for five days during the winter in a hot room kept by an air conditioning unit (for which we are indebted to the General Electric Company) at 32.5° (36 per cent humidity) during the day and at 31° at night. The room then was cooled for thirty hours; subject A submitted himself to a second period of heat for four days. In subject A the serum volume was increased 7 per cent above the control value after one day of exposure to heat, 29 per cent after five days exposure; and 28 per cent after four days exposure in a second period of heating. In subject B the increase was 13 per cent in two days and 29 per cent in five days. At the time of increased serum volume the concentration of serum proteins (as estimated by specific gravity) remained approximately normal; the total serum protein of the body was therefore greater. The total cell volume increased with the serum volume but not proportionately. In both subjects increase in blood volume of approximately one liter was associated with increase of body weight of about one kilogram.

*The effect of salts on the diabetes insipidus following post-hypophysectomy in the rat.* H. G. SWANN and B. J. PENNER (introduced by A. J. Carlson). Department of Physiology, University of Chicago, Ill.

In the rat after removal of the "post-hypophysis" (removal of pars nervosa and pars intermedia, with the pars anterior left intact), the ensuing diabetes insipidus persists for approximately two months. The administration of NaCl in the drinking water exacerbates the condition considerably, the animals excreting above five times more urine than

normal rats drinking the same solutions. Such treatment prevents the spontaneous disappearance of the diabetes insipidus. For example, two post-hypophysectomized rats have been kept diuretic for nine months by NaCl administration. In these rats, the diabetes insipidus slowly becomes worse; at times, a 190 gram rat may secrete 100 c.c. or more of urine per day, whereas the normal rat under these conditions secretes 8 c.c. per day.

Pituitrin prevents these saline diureses.

Ringer's solution and $NaHCO_3$ also exert diuretic effects, but Na citrate, $Na_2HPO_4$, $NaH_2PO_4$, KCl, and $CaCl_2$ are handled normally.

*Cinephotography of the pupil.* S. A. TALBOT (introduced by W. H. Marshall). Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital, Baltimore, Md. (Demonstration.)

Infra-red illumination permits cinephotography of pupils dilated to 6 mm. or more, and enables studying the reflex motion of light or dark irises under various conditions of adaptation and stimulation. The eye can see infra-red light far beyond available film-sensitization, so that conditions of least luminosity must be used. A rebuilt Zeiss camera records 5 frames per inch, at f:2 aperture. A speed of 16 exposures per second resolves details of latency and the initial transient response. Stringent precautions are needed to exclude the psychodilation of the pupil, and so obtain a good baseline. For this purpose, acoustic shielding and an improved headrest are used.

The apparatus is adapted to many sorts of experiment, on an individual or comparative scale. The brightness, area, locus and duration of the stimulus are accurately controllable through a wide range. Convergence and retinal adaptive level may be adjusted at will. Latency of pupillary contraction, which varies between 0.20 and 0.35 sec with experimental conditions, is made measurable to a precision of 0.03 sec by synchronizing the camera and flasher. A technic exhibited gives the pupillograms a diametral precision of 0.1 mm.

Reproducible behavior of the pupil is demonstrated by progressive families of response-curves. These show a number of reliable features, which vary systematically with stimulating conditions. The reciprocity law holds for the energy evoking a given amplitude. A relation between pupillary response and stimulus strength is shown for a dark-adapted eye. Other visual functions are paralleled objectively.

*Direct-coupled amplifier.* S. A. TALBOT (introduced by W. H. Marshall). Wilmer Ophthalmological Institute of the Johns Hopkins University and Hospital, Baltimore, Md. (Demonstration.)

A compact and stable differential amplifier operates a 2000v cathode-ray tube at a useful sensitivity of 2 microvolts/mm, or 0.4 microvolts/mm maximum. The frequency characteristic is essentially linear from zero to 10 kc. The tubes need not be matched statically or dynamically. Besides the a.c. line, a 6v storage battery and 67v of B supply are used.

Cathode-bias resistors supply all the interstage matching voltages, instead of coupling batteries. The degenerative action of these resistors makes the gain of the common mode so small in comparison to that of the signal (differential mode) that the operating points are quite stable. An indicator tube enables adjustment and check on the operating points of all

four stages. This device preserves linearity of deflection and focus of the trace. Interaction of common and differential mode is eliminated by a single control, so that a 10 mv common signal may be impressed on the input without effect, at a differential sensitivity of 2 microvolts/mm. Controls are provided also for calibration signal, horizontal and vertical zero, gain, and change to capacity coupling at any desired decrement. Neither the initial balance nor its maintenance require expert operation.

*The energy cost of standing.* RUBYE H. TEPPER (by invitation) and FRANCES A. HELLEBRANDT. University of Wisconsin, Madison. (Read by title.)

Since standing consists of movement upon a stationary base, a measurable quantity of energy is probably used for the performance of work. The object of this study was to determine the range of the metabolic increase due to the maintenance of a passively assumed standing posture. Total metabolism was measured by indirect calorimetry in 75 young adult women in recumbency and during five minutes of comfortable standing. The frequency distribution of the basal metabolism was approximately normal, the mean metabolic rate being 35.1 cals./sq.m./hr., $\pm 0.251$ with a standard deviation of $\pm 3.21$. The average increase on standing was 5.24 cals./sq.m./hr., the relative value being 16.3 per cent with a range of 0.90 per cent to 50 per cent. It is suggested that the wide variance in metabolic increases may be related to the steadiness of the stance. To evaluate this hypothesis, the metabolic rate is now being determined in a relaxed, motionless vertical posture with the subject suspended in water, in supported postures on land with the subject held at various angles between the horizonal and the vertical, and in free standing during which the postural stability is quantitatively measured by continuously recording shifts in the center of weight. A series of 10 preliminary experiments comprising 107 metabolic rate determinations have been performed on 5 subjects. Suspension in water induces a small mean metabolic increase within a narrow range. There is no appreciable augmentation in metabolism at graded vertical postures until a 75 degree angle is reached. The preliminary observations do not warrant any conclusions concerning the relation of the degree of metabolic stimulation to the magnitude of natural and experimentally induced variations in postural sway.

*Differential manometer records of gastric and duodenal pressures.* J. EARL THOMAS. Jefferson Medical College, Philadelphia.

Comparison of the intragastric with the intraduodenal pressure by means of a differential manometer yields significant information regarding the forces that effect gastric emptying. The results are superior to those obtained with simple manometers because they are not complicated by intra-abdominal pressure changes.

Observations were made on fistula dogs while the stomach was emptying various types of meals and during the action of HCl and peptone solutions in the duodenum. The manometer was connected to tubes whose open ends lay within the stomach and duodenum. Balloons were not used.

When the stomach contains food or liquid the intragastric pressure near the pylorus is generally slightly higher than the pressure in the first part of the duodenum, even at times of relative inactivity. Cyclic changes in

pressure relations occur, presumably associated with gastric and duodenal peristalsis. A complete cycle consists of the following three phases: (1) the excess of gastric over duodenal pressure increases slowly to a maximum: (2) the excess of gastric pressure diminishes rapidly until the pressures are approximately equal; (3) the duodenal pressure rises rapidly until it greatly exceeds the gastric pressure and then falls, generally to a level below the gastric pressure. The first two phases result mainly from changes in the gastric pressure; the third phase is caused exclusively by changes in the duodenal pressure.

Incomplete cycles, characterized by the absence of one or more of the three phases, are common. The phase most frequently absent is the primary increase in the excess of gastric over duodenal pressure. This phase is, apparently, not essential for gastric emptying. It is nearly always absent during the emptying of liquids, which leave the stomach rapidly. It is more likely to be present when the stomach is dealing with solid food and the emptying is, consequently, relatively slow.

Injection into the duodenum of HCl or peptone solutions brings about an equalization of gastric and duodenal pressures and a temporary cessation of the cyclic changes. Evidently the influence of these agents on gastric emptying, under these circumstances, is independent of any influence they may have on the pyloric sphincter.

*Non-species-specificity of gonadotropic antihormone.*[1] K. W. THOMPSON[2] (introduced by Harvey Cushing). Department of Surgery and the Laboratory of Physiological Chemistry, Yale School of Medicine, New Haven, Conn.

The development of non-species-specific gonadotropic antihormones in the sera of two dogs and a horse was observed during 15 months of daily injections of an extract of sheep-pituitary glands. At intervals during the period the animals' sera were studied for ability to inactivate in test-rats extracts from both sheep and human pituitary glands. A practical source of anti-human-pituitary serum from horses was sought.

The first observed effect in the rats was an augmentation of the gonadotropic action. After 100 days this activity of the sera of the horse and dog No. 1 disappeared in respect to the sheep-pituitary extract, but the augmentation continued with respect to the human hormone.

The 200-day serum of dog 1 was the first to show signs of inactivating the human material. This particular action increased and became highly effective thereafter.

On the 250th and 280th days the horse's serum had a slight anti-human effect, which was greater on the 14th day after a maximal bleeding. After the 350th day no anti-human action was detectable.

The serum of dog 2 acquired demonstrable anti-sheep effects after the 200th day. It continued to augment the human material and never showed an anti-human effect.

An attempt was then made to hasten the development of the desired effect in two other dogs by injecting a mixture of sheep-pituitary extract which had been incubated with 10 per cent swine serum. This procedure

[1] Aided by grants from the Josiah Macy Jr. Foundation and from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry.

[2] Alexander Brown Coxe Fellow.

hastened the anti-hormone production; the human hormone was effectively inactivated after the 60th day by both animals' sera.

The results indicate that although the treatment given the horse did not offer a practical method of producing antihormones for human material, they did lend further support to the theory that anti-hormone are immune bodies.

*The sodium and chloride retaining effect of compounds derived from the adrenal cortex.* GEORGE W. THORN (by invitation), ARTHUR GROLLMAN and LEWIS L. ENGEL (by invitation). Chemical Division of the Medical Clinic and the Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University and Hospital, Baltimore, Md.

Earlier studies (Proc. Soc. Exper. Biol. and Med. **36:** 361, 1937; J. Exper. Med. **65:** 757, 1937) have indicated that, in normal human subjects and normal dogs, injections of adrenal cortical extract induce a sodium and chloride retention. This retention of sodium and chloride has been shown, however, not to be specific for adrenal cortical extracts but is also exhibited, by several of the sex hormones (Science **86:** 40, 1937). We have, therefore, investigated various extracts and crystalline compounds derived from the adrenal cortex to determine:

1, which of the compounds derived from the adrenal cortex produce sodium and chloride retentions;

2, to what extent the hormonal activity (as measured by the ability of the preparation to maintain adrenalectomized dogs and rats) of extracts and compounds derived from the adrenal cortex parallels their sodium retaining effect.

These studies contribute to our understanding of the action of the adrenal cortical hormone and also have a direct bearing on the interpretation of assays of the hormone carried out by the usual methods (*cf.* Cold Spring Harbor Symposia on Quantitative Biology, **5:** 313, 1937).

*Electrolyte changes in cat muscle during activity.* S. R. TIPTON (introduced by F. A. Hitchcock). Department of Physiology, Ohio State University, Columbus.

Water and electrolyte content were determined on one gastrocnemius muscle after stimulation for thirty minutes, the other gastrocnemius being used as control. The essential changes in cat skeletal muscle during activity are a loss of magnesium and potassium and a gain of water, sodium chloride and calcium. A small part of the sodium goes inside the cell to exchange with K. The longer the muscle is stimulated, at least up to three hours, the greater the electrolyte exchange on the basis of the dry weight of the muscle. After prolonged stimulation the water content of the active muscle may even be less than the control. Curves are plotted for recovery changes in 1 hour, 2 hours and 3 hours after stimulation. After three hours there is about 90 per cent restoration of the K lost during activity, 85 per cent of the water gained has left the muscle and about a 45 per cent loss of the sodium taken up during activity.

Continuous injection of 1 per cent $CaCl_2$ until plasma Ca was raised about 20 per cent caused about 5 per cent reduction of the tension exerted by the muscle but had no appreciable effect upon changes during activity. Recovery changes appeared to be slowed slightly. One per cent KCl also had no effect upon activity changes but there is a distinct suggestion of a speeding up of recovery changes.

Cortin was injected subcutaneously into two normal cats in larger than maintenance doses for three successive days, following which one muscle was stimulated and both muscles analysed for electrolyte changes. Plasma sodium and chloride concentration was increased over that of the normal animal but the exchange during activity and subsequent recovery were not affected appreciably.

*The spontaneous hemostasis of small wounds. The pressure required to make a closed incision of the skin to resume bleeding.*[1] L. M. TOCANTINS (introduced by A. Cantarow). Department of Medicine, Jefferson Medical College, Philadelphia.

Incisions of uniform dimensions were made in the abdominal skin of dogs. At varying intervals after cessation of the bleeding, negative pressure, developed at a uniform rate, was applied to the wound until blood reappeared. The pressure required to produce this effect (hemostatic pressure) varies directly as the incision ages. Significantly lower values are found in thrombopenic dogs with a prolonged bleeding time, and in traumatized or infected incisions. Abnormally high values may be found in dogs with a short bleeding time, hypercoagulable blood and a thrombocytosis. In estimating the factors that prevent the recurrence of bleeding from an incision in the skin, the physical properties of the clot, the contractility of the skin vessels and the resistance of the skin must be taken into account.

*Reflex discharges from the spinal cord over the dorsal roots.* J. F. TOENNIES (introduced by H. S. Gasser). Laboratories of The Rockefeller Institute for Medical Research, New York City.

Leads from the saphenous nerve show that at a constant interval following an afferent volley in the same nerve (or in other nerves of the same or the opposite limb) there occurs a large discharge of centrifugally conducted impulses lasting about 20 msec. The reflex starts when threshold fibers in the centripetally conducting portion of the arc are stimulated, and the discharge increases as the number of fibers in the centripetal volley is increased. The first reflex impulses to be recorded are carried in the fastest fibers in the nerve (80 m.p.s.). Fibers of other velocities are also involved and often a detached group can be shown to be travelling at delta velocity.

Similar reflex discharges can be obtained in leads from the lumbar and sacral dorsal roots when homolateral or contralateral roots or nerves are stimulated. The reflex time measured from the beginning of the entrance of the volley into the spinal cord to the beginning of the discharge is 3.5–4.5 msec. for the homolateral reflex and 0.7–2.0 msec. longer for the contralateral reflex. The time is independent of the strength of the exciting volley, but the duration of the discharge is increased by the larger volleys. No discharge occurred early enough to be interpreted as taking place in recurrent fibers as described by Barron and Matthews (J. Physiol. **85:** 73, 104, 1935).

The dorsal root reflex exhibits properties similar to a ventral root reflex. Spatial summation can be effected by simultaneous stimulation of two roots. Conditioning of the cord by one volley inhibits the effect of a sub-

[1] Aided by a grant from the J. Ewing Mears Research Fund.

sequent volley for more than one second; and the course of the inhibition parallels that of the ventral root reflex and the curve of the recovery of responsiveness in the internuncial neurons. Tetanic stimulation produces a "jet" type reflex in the dorsal roots, the rate of decay of the successive discharges increasing with the frequency of the exciting stimuli. Asphyxia promptly abolishes the reflex.

*Pyramidal and extrapyramidal factors in the production of proprioceptive reflex grasping in the monkey.* SARAH S. TOWER. Department of Anatomy, Johns Hopkins University, Baltimore, Md.

Reflex tonic grasping in response to stretching the flexor muscles of the fingers is now widely considered pathognomonic of lesion in Brodmann's cortical area 6. However, at least one other lesion produces a similar, though simpler and slighter result.

Bilateral section of the pyramidal tracts at the medullary level in the adult monkey or uni- or bilateral section in the newborn, while eliminating contact grasping, produces proprioceptive reflex grasping in the digits affected. On the other hand, unilateral section in the adult produces a condition of hypotonia and minimal usage of the opposite extremities in which grasping of any sort is little apparent. The reflex tonic grasping of pyramidal tract lesion is most easily understood as inability to open the hand voluntarily due to paresis of the extensors of the fingers. From these results it might be surmised that the similar grasping from area 6 lesion represents inability to operate through the pyramidal pathway.

However, after complete bilateral section of the pyramidal tracts, performed acutely at the medullary level, electrical stimulation of area 6 relaxes such reflex tonic grasping as may be obtained under light ether anaesthesia. Hence this area undoubtedly possesses an extrapyramidal mechanism capable of relaxing a grasp. The cortex therefore has in its control at least a duality of mechanisms for releasing grasp. Why these are so poorly able to operate one for the other, presents, a problem, probably in intracortical function, which the observations do not solve.

*Respiratory and circulatory changes resulting from artificially ventilating the two lungs in and out of phase.* ROBERT TRIMBY (by invitation) and HAYDEN NICHOLSON. Department of Physiology, University of Michigan, Ann Arbor.

These experiments were performed upon dogs anaesthetized with morphine and urethane. A cannula was inserted into the left bronchus through the trachea. The cannula was provided with a rubber cuff, inflation of which completely closed the bronchus proximal to the opening of the cannula. A second cannula was inserted into the trachea almost to the bifurcation. The chest was opened on either side and the two lungs were ventilated artificially by individual pumps, the stroke and rate of which could be varied. Changes in volume of each lung were recorded from spirometers and changes in pressure by tambours. Arterial blood pressure was recorded by a mercury manometer.

With the pumps operating at slightly different rates and at sufficient volume to produce apnea, the two records of intra-pulmonic pressure showed slight waves in amplitude, the crest of each wave occurring when respiration in the two lungs was in phase, the trough when the two were completely out of phase. In experiments in which the ventilation was not

sufficient to produce apnea, pressure waves were again observed, the lowest pressure occurring when the inflation by the pump coincided with the animal's inspiratory movement.

When the animal was in apnea and the two pumps in phase, the blood pressure tracing showed typical cardio-respiratory waves, the rise in pressure beginning shortly after the onset of inspiration, the fall shortly after the beginning of expiration. As the inflation of the two lungs occurred farther and farther out of phase, the blood pressure waves gradually diminished in amplitude and finally when the two lungs were exactly out of phase, the waves completely disappeared. We believe this observation throws some light on the mechanisms responsible for the production of cardio-respiratory waves.

We are now carrying on experiments similar to the above in which the inflation of the lungs is accomplished by negative rather than positive pressure.

*Responses of normal and hypophysectomized immature rats to injections of a menopause urine extract.* H. H. TYNDALE (by invitation), LOUIS LEVIN (by invitation), and P. E. SMITH. Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City.

Immature hypophysectomized rats, as compared with normal rats, show a marked difference in ovarian response to menopause urine extract treatment. Over a wide dose range, corpora lutea appear in many of the ovaries of normal but not of hypophysectomized rats, although the ovarian weights in the latter are at least as great as in the normal animals. With extremely high doses, luteinization takes place in the hypophysectomized rats also. To produce the same degree of luteinization, with the preparation used, doses ten to twelve times those required by the normal animal must be given. This indicates that the pituitary gland plays an important rôle in the luteinization of the ovary of the normal, menopause-urine-treated rat.

In the hypophysectomized rats, the occurrence and degree of luteinization is correlated with the dosage. In the normal rats corpora lutea appear, at intermediate dose levels, in a variable percentage of the ovaries, while the remaining ovaries at a given dose show marked follicular enlargement, but no lutein changes. When corpora are present the ovaries attain a much greater average weight. This results in an enormous degree of individual variation in the ovarian weight response. As a consequence, the ovary of the normal immature rat is highly impractical for assay of menopause urine. A prepubertal pituitary ovarian cycle is postulated to explain the marked individual difference in the response of normal immature rats on the same dose of menopause urine.

*The toxicity and pharmacology of nicotinic acid.* KLAUS UNNA (introduced by H. Molitor). Merck Institute of Therapeutic Research, Rahway, N. J.

The lack of experimental data on the pharmacology and toxicology of nicotinic acid, recently shown to be of value in the treatment of human pellagra, prompted the following investigation. Because of the low solubility and strong acid reaction of pure nicotinic acid, most of the following experiments were made using a solution of sodium nicotinate (pH 6.9–7.1) equivalent to 10 per cent nicotinic acid.

*Acute toxicity.* The toxicity was studied in mice, rats and dogs. All animals were kept on a complete diet and under controlled temperature and humidity conditions. The minimum lethal dose for rats and mice approximates 5 grams per kilogram subcutaneously and 7.5 grams per kilogram orally. In dogs one gram per kilogram was tolerated without toxic symptoms. Since the injection of equal volumes of equimolecular concentrations of sodium chloride and sodium nicotinate solutions produce deaths with similar symptoms, it may be assumed that the lethal effect of these high doses is caused by physical factors rather than by a specific toxic action of nicotinic acid.

*Chronic toxicity.* A group of young rats were given orally over a period of 40 days 1 gram per kilogram nicotinic acid daily. During this period the animals showed approximately the same gain of weight as the controls. No toxic symptoms were observed. The histological examination failed to show any pathological changes.

*Pharmacology.* In a series of 22 experiments on rats receiving orally or subcutaneously doses from 0.5 to 2.0 grams nicotinic acid per kilogram no changes in oxygen uptake as determined by the Richards and Collison method was observed in experiments of 5 hours duration. The blood pressure of rabbits and cats was not influenced by the intravenous injection of one gram nicotinic acid per kilogram. The electrocardiogram showed no change in heart action. No change in the diameter of the peripheral vessels nor in skin temperature was observed following the injection of large doses of nicotinic acid.

*Effect of vitamin C on the contraction height of the frog heart.* FRANK URBAN and HUBERT B. PEUGNET (introduced by Joseph Erlanger). Departments of Biological Chemistry and Physiology, Washington University School of Medicine, St. Louis. (Read by title.)

Isolated hearts of Rana pipiens were perfused at room temperature with bicarbonate Ringer's, according to the Straub technique. A continuous stream of 95 per cent $O_2$ + 5 per cent $CO_2$ was passed through the solution in the cannula, to maintain oxygen saturation and pH. Vitamin C was added as the sodium salt, in amounts to give concentrations of 1–30 mgm. of vitamin C per 100 ccs. of Ringer's. Concentrations very much higher may be toxic. The amount of the improvement with vitamin C varied from a small increase in fresh heart to an increase of several hundred per cent in the long perfused heart. The effect reached its maximum in 1–5 minutes after introduction of vitamin C. The positive inotropic action of vitamin C was not abolished by changes in the calcium and potassium concentration, over a wide range. The duration of the effect was a prolonged one; it was only slowly reversible on washing. The vitamin C effect was independent of the presence or absence of glucose in Ringer's solution.

*The effect of anoxia on reflex dilatation of the pupil.* BORIS URY (by invitation) and ERNST GELLHORN. Department of Physiology, College of Medicine, University of Illinois, Chicago. (Read by title.)

Experiments werec arried out in rabbits under light urethane anesthesia. The pupillary reaction was studied either in the normal or in the sympathectomized eye. The sciatic nerve was stimulated with weak faradic currents for 1–2 seconds and the pupillary reaction was measured with an ocular micrometer. The threshold for the pupillary dilatation fol-

lowing stimulation of the sciatic nerve is the same for the normal and sympathectomized eye indicating that under the condition specified the reaction involves no sympathetic stimulation but only parasympathetic inhibition. It occurs in absence of vocalization and reflex movements which may be observed when supraliminal stimuli are used. When 6–8 per cent oxygen is inhaled the threshold is raised. This process is reversible upon readmission of air. The effect of 6 per cent oxygen can be completely offset by 5 per cent $CO_2$ so that no change in threshold occurs. Five per cent $CO_2$ itself is without effect on the reaction studied. The experiments show that the effect of oxygen deficiency on inhibitory processes is similar to that previously studied by Gellhorn and collaborators (cf. Sigma Xi Quart. **25:** 156, 1937) in regard to excitatory cortical and subcortical processes.

*The effect of acute hemorrhage on absorption from the small intestine.* Edward J. Van Liere, David Northup and Clark K. Sleeth (by invitation). Department of Physiology, West Virginia University, Morgantown.

Dogs were used which had been given barbital intravenoulsy. They had been starved 48 hours previously and were paired as near the same weight and age as possible. One animal was used as a control; the other was bled 3.2 per cent of its body weight. The intestines were exposed by a midline incision. With the exception of the duodenum the entire small intestine was used as a loop. The length of the loops were made identical by actual measurement. The effect of hemorrhage on absorption of water, normal salt solution and isotonic glucose solution was studied. Each substance was used separately. Ten pairs of animals were used when absorption of water was studied; the same number were used when sodium chloride solution was placed in the small intestine. Fifteen pairs, however, were used when the absorption of glucose was studied, so 70 animals were used in all.

A measured amount of the substance to be studied was placed in the loop; the same amount was used in the control as in the experimental animal. The distilled water was left in the intestine for 30 minutes, salt solution for 40 minutes and glucose for 90 minutes. At the end of the allotted time the contents of the intestine were removed and measured. The actual content of the solute present was determined by the Van Slyke modification of the Vollhard method for the chloride and by the Folin-Wu method for the glucose.

It was found that the control animals absorbed more water from the small intestine than did those which had sustained a hemorrhage. The opposite was true in the studies made on the absorption of normal salt solution. The absorption of isotonic glucose solution was not affected by hemorrhage.

*Failure of continued injections of gonadotropic hormones to prevent menstrual cycles and pregnancy in Macaca mulatta.* G. Van Wagenen and H. H. Cole (by invitation). Department of Obstetrics and Gynecology, Yale University Medical School, and University of California, Davis. (Read by title.)

Mare serum when injected daily into immature monkeys for 50 to 70 days has been shown to cause an activation of the ovaries with sex skin

swelling and a single menstruation after which the ovaries regressed to infantile size and the blood sera were found to have a strong inhibitory action against the gonad-stimulating hormones of pregnant mares (Meyer and Gustus, Science: 81, 208). In extending these observations we have injected three female macaque monkeys of prepuberal status (2500–2850 gm.) with 35, 75 and 400 R.U. of pregnant mare serum daily for six to eight and one half months. The ovaries were stimulated to cystic hypertrophy and menstruation followed in 19, 22 and 29 days, respectively. Following the initial activation the sex skin faded, the cystic condition receded and there were quiescent periods of 115, 58 and 170 days after which regular cycles were established in spite of the continued injections.

Pregnant mare serum injections into various animals are reported to give rise to serological substances which are effective against the action of the gonadotropic hormones of the serum. In this series it is clear that, although an undoubtedly high titer of anti-gondaotropic substances existed in the sera of the monkeys injected, it was not effective in inhibiting the action of the animals' own hypophysis. Nor were there any injurious effects upon the ovaries because two of these animals which were mated shortly after cessation of injections became pregnant.

*A study of the metabolism of progesterone.* ELEANOR H. VENNING (by invitation) and J. S. L. BROWNE. McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

The excretion of pregnanediol in the form of sodium pregnanediol glucuronidate following injections of progesterone has been studied after hysterectomy and ovariectomy during the normal menstrual cycle and in pregnancy; also in cases showing endometrial hypoplasia and hyperplasia. Whenever possible endometrial biopsies have been taken and the corresponding changes in the endometrium observed. The effect of a preliminary treatment with oestrin as well as the simultaneous injection of oestrin and pregnancy urine extracts has also been investigated.

In two cases after hysterectomy no pregnanediol was excreted following the injection of 24 mgm. progesterone. In these cases 60 mgm. sodium pregnanediol glucuronidate were also injected in aqueous solution and 41–58 per cent was recovered. In one case after ovariectomy 12 per cent of injected progesterone was recovered as pregnanediol. When progesterone was injected during the luteal phase of the normal menstrual cycle 20–30 per cent was recovered as pregnanediol, when pregnancy urine extracts were injected simultaneously, the recovery was 42–70 per cent.

In cases of hypoplasia a pretreatment with oestrin favoured the output of pregnanediol. Pregnancy urine extracts when given with the progesterone appeared in some cases to increase the output. The cases of hyperplasia gave variable results. In one case 110 mgm. were injected over a period of 11 days. No pregnanediol was excreted for the first three days and about 1 mgm. per day for the last 8 days.

The study of these findings suggest that the excretion of pregnanediol is dependent not only upon the presence and amount of progesterone, but also upon other factors as yet not clearly defined, possibly 1, state of the endometrium; 2, amount of oestrin present; 3, efficiency of glucuronide conjugation mechanism; 4, renal excretion and 5, excretion of pregnanediol in a form other than the glucuronidate.

It is a difficult matter to know how much of the injected hormone reaches its site of action and the very low amounts of pregnanediol recovered in most of the cases might also be due to faulty absorption or to destruction of the hormone.

*The visual purple system in fresh-water fishes.* GEORGE WALD. Biological Laboratories, Harvard University, Cambridge, Mass.

The visual purple in the rods of mammals, frogs and marine fishes (rhodopsin) absorbs light maximally at 500 mμ and participates in a retinal cycle of the form, rhodopsin $\overset{\text{light}}{\rightleftharpoons}$ retinene + protein → vitamin A + protein → rhodopsin. The visual purple of fresh-water fishes (porphyropsin) absorbs light maximally at 522–525 mμ. It participates in a system similar to that of rhodopsin, but with the spectra of all components displaced about 20 mμ toward longer wave-lengths (*Wald, Nature,* **139**, 1017, 1937).

I have examined the porphyropsin cycle in retinas of white perch (*Morone americana*), pickerel (*Esox reticulatus*), and calico bass (*Pomoxis sparoides*). No vitamin A or retinene occurs in these tissues.

Benzine extracts of dark adapted retinas contain small quantities of light yellow pigment. Tested with antimony trichloride this displays a faint blue color due to a low absorption band at 696 mμ.

Subsequent exposure of the same retinas to bright light bleaches them from purple to russet. Immediate re-extraction with benzine yields a large quantity of golden pigment, liberated in bleaching. This possesses a single absorption band in chloroform at about 404 mμ, and yields with antimony trichloride a high band at 703 mμ. It is homologous with retinene in the rhodopsin system.

Following irradiation, retinas fade slowly in light or darkness from russet to pale yellow. Benzine extracts of completely faded retinas contain no 703 mμ-chromogen, but instead a large quantity of newly arisen light yellow pigment. This possesses a single band in chloroform at about 354 mμ, and yields with antimony trichloride a high band at 696 mμ. It is homologous with vitamin A in the rhodopsin system.

Large quantities of this substance are found also in the pigment epithelium of the eye, and in the livers of these and European fresh-water fishes (see Lederer, Rosanova, Gillam and Heilbron, *Nature* **140**: 233, 1937). In both tissues it pre-empts the position occupied by vitamin A in other vertebrates. Because of its retinal activity it has been proposed to call this substance vitamin $A_2$. Its retinal precursor might correspondingly be designated retinene$_2$. The porphyropsin system may tentatively be formulated: porphyropsin $\overset{\text{light}}{\rightleftharpoons}$ retinene$_2$ + − − − → vitamin $A_2$ + − − − → porphyropsin.

*Experiments on water diuresis.* ARTHUR M. WALKER. Laboratory of Pharmacology, University of Pennsylvania, Philadelphia.

The experiments relate to the control which the posterior pituitary is believed to exert upon normal processes of water excretion by the kidney. It has been found that Pituitrin or Pitressin, added to rats' urine, is largely lost from the urine by dialysis through thin cellophane against running water for 3 hours; that small but effective amounts of Pituitrin added to rats' urine of pH 5.5 or higher become ineffective on standing 24 hours;

that Pituitrin, injected subcutaneously in rats, is not excreted in the urine unless relatively enormous doses are used; that a colloidal substance having an antidiuretic action which in some respects does not resemble that of Pituitrin is present in the urine of normal rats and rabbits and in that of completely pituitarectomized rats. The amount of this substance excreted is not increased conspicuously when the animals are deprived of water. The experiments have thus far failed to show that the antidiuretic principle of the pituitary is present in the blood and urine of either hydrated or dehydrated animals.

Water, subcutaneously injected into rats, produces diuresis; similar amounts of isotonic NaCl are without effect; hypertonic sodium chloride or glucose solutions produce antidiuresis.

*Digital blood pressure changes after insulin.* ORVILLE S. WALTERS and ALRICK B. HERTZMAN. Department of Physiology, St. Louis University School of Medicine, St. Louis, Mo.

A modification of the method of Gaertner was used to estimate digital blood pressure. The Gaertner capsule was employed, but the exact time of passage of blood through the sleeve was indicated by interposing the finger tip between a source of light and a photo-electric cell connected with an amplification system.

Serial observations every 2 minutes were made on the brachial and digital pressures of 10 fasting normal men. After a control period of 30 minutes, each subject was given a 10-unit intravenous injection of insulin and the pressures were followed for 90 minutes after its administration.

Since the digital pressures are more variable than brachial, the experimental period of 120 minutes was divided into three 40-minute periods for analysis. The effects of the insulin were most marked during the second 40-minute period.

The mean of the brachial pressure readings for the second phase showed an increase in 9 of the 10 subjects, varying from 3.6 to 21 mm. rise. The mean of the digital pressure readings showed an increase during the second period in only 5 of the 10 subjects, varying from 5 to 12 mm. rise. The brachial-digital difference increased during the second phase in 9 cases in amounts varying between 2.8 and 13.6 mm. in the different subjects.

Additional studies made on subjects given intramuscular injections of 0.5 cc. 1:1000 adrenalin showed similar effects, the increase in brachial-digital difference paralleling the intensity of response to adrenalin.

*Intestinal obstruction and plasma volume.* C. B. WELD. Department of Physiology, Dalhousie University, Halifax, N. S.

The plasma volumes were determined by a modification of the Brilliant Vital Red dye method. (Weld and Woodward, J. Lab. Clin. Med. **22:** 410, 1937.) A further improvement used in these experiments was the addition of a deep blue color filter to the eyepiece of the colorimeter. This still further reduces the error caused by a possible small amount of hemolysis in the plasma.

Approximately equal degrees of reduction in plasma volume (in the order of 40 per cent of the original plasma volume) were induced in two groups of dogs. One group had a "Whipple Closed Loop" operation performed on the previous day; the animals appeared well though somewhat apathetic, vomiting had not yet become severe, the arterial pressure was

about 70 mm. Hg, and the hemoglobin was increased by 15–25 per cent. The other group had merely been deprived of drinking water for two or three days; the blood pressure of these animals was about 100 mm. Hg and their hemoglobin was unchanged or only slightly raised.

These dogs were then bled, 20–25 per cent of their original blood volume being removed by cardiac puncture, with no anaesthetic. The water deprived dogs tolerated the hemorrhage well. The obstruction dogs on the other hand, died almost at once, some even dying before all the blood had been withdrawn.

It is suggested that these findings are further evidence that anhydremia and dehydration are not the sole cause of the prostration and death in acute high intestinal obstruction.

*Adaptation of Bingham type of viscometer to small amounts of blood serum.* H. L. WHITE. Department of Physiology, Washington University School of Medicine, St. Louis, Mo. (Demonstration.)

Accurate viscosity measurements afford probably the best means of detecting small and acutely produced changes in serum protein content. The "micro" adaptation of the Bingham type of viscometer permits determinations of relative viscosity on samples of a few tenths of a cubic centimeter with an error not greater than $\pm 0.1$ per cent. Since the relative viscosity of normal human or dog serum usually is between 1.5 and 1.8, values for relative viscosity should have an error not greater than $\pm 0.002$. A change of 0.002 in relative viscosity represents a change of approximately 0.014 gram per 100 cc. in protein content, which for most serums or plasmas will be a change of about 0.2 per cent.

The errors of the method are less than are those usually inherent in the collection of blood samples, i.e., duplicate determinations on a given sample will check within closer limits than will determinations on two samples drawn a few minutes apart. However, subjects from whom blood can be drawn without stasis will consistently show about two hours after drinking 1200 to 1500 cc. of water a drop in serum viscosity corresponding to a drop of 0.04 to 0.1 gram per 100 cc. in protein content. This is followed by a rise above the normal level such that at $3\frac{1}{2}$ to 4 hours after the drink the serum protein content is increased above the initial value by 0.15 to 0.20 gram per 100 cc. Much greater drops in viscosity are seen after salt solution by mouth or after water with pitressin.

Two runs are taken in each direction. Pressures of 600 to 1000 mm. water are used; the maximum fluctuations during a determination are not more than 0.3 mm. Temperature is maintained at 25°C. $\pm 0.002$. Transfer time is always greater than 200 seconds. The capillary in the instrument shown has a bore of 0.20 mm.

*Failure of normal diuretic response in a case of diabetes insipidus surgically produced.* H. L. WHITE. Department of Physiology, Washington University School of Medicine, St. Louis, Mo. (Read by title.)

A patient of Dr. Ernest Sachs in whom a high polyuria immediately followed craniotomy for removal of a suprasellar cyst showed on 3 occasions 3 to 4 weeks postoperatively no diuretic response within several hours to drinking 3.9 per cent body weight of water within a 10 to 15 minute period; she was forming urine at about half her known maximum rate prior to the water administration but was not dehydrated. This

result was unexpected since the lesion was presumably the same as that which produces permanent polyuria in dogs, which do show a normal response to comparable water administration. She behaved rather as did our observed human cases of spontaneously occurring diabetes insipidus. No check on the nature of the lesion is available, since the patient is living.

*Hypophyseal influences on renal function and water exchange in the dog.* H. L. WHITE and PETER HEINBECKER. Departments of Physiology and of Surgery, Washington University School of Medicine, St. Louis, Mo.

All remarks apply to dogs. High polyuria means 5 to 10 times normal output.

The polyurias of hypophyseal stalk section and of hypophysectomy are transitory (2 days to 2 weeks) and usually immediately abolished by thyroidectomy. The high polyuria of postlobectomy persists several weeks, gradually declining. Polyuria produced by interruption of the supraoptic-hypophyseal tract plus destruction of the tuber cinereum is high and permanent; it is not affected by subsequent hypophysectomy or by hypophysectomy plus thyroidectomy. It is postulated that a necessary and sufficient condition for permanent high polyuria is removal (anatomical or functional) of pars nervosa and of accessory potential sites of pitressin production.

Dogs with permanent polyuria show an early (2 to 4 weeks postoperative) fall of 30 to 50 per cent in urea and creatinine clearances, rise of 100 per cent in blood urea, more variable but not consistently altered serum electrolyte content, no albuminuria. After several months these values are returning toward normal. The same is true of hypophysectomized dogs.

Maximum urine concentrating power (after 24 hours' water deprivation) of hypophysectomized dogs is lowered about 25 per cent with respect to creatinine, about 50 per cent with urea and unchanged with chloride. Approximately comparable results are obtained with permanently polyuric dogs. Rates of urine flow on deprivation are slightly greater after hypophysectomy than before, with a somewhat greater increase with permanently polyuric dogs.

Within the first few days after hypophysectomy the diuretic response to 3 per cent body weight of water may be slight. A month postoperative and thereafter the response is normal with respect to its time relations but may not reach so high a crest as preoperatively. Permanently polyuric dogs show a normal response to 3 per cent body weight of water, both temporally and quantitatively. Blood dilution with both classes is normal when normal urine curves are obtained; greater when diuresis is subnormal (hypophysectomized). There is no evidence that normal diuretic response to water depends upon the integrity of the posterior lobe or that the response is quicker in its absence; ability to conserve water on deprivation is, however, impaired.

*Further study on threshold of knee-jerk as index to physical fitness.* G. C. WICKWIRE (by invitation), H. L. TERRY (by invitation), RUTH KROUSE (by invitation), W. E. BURGE and C. D. MONSSON (by invitation). Department of Physiology and School of Physical Education, University of Illinois, Urbana. (Read by title.)

The arc through which the hammer swung to strike the patella ligament to produce the knee-kick was kept constant while the weight of the hammer and hence the strength of the stimulus was varied by adding or removing weights from a carrier attached to the hammer.

Upon arising at seven A.M. it required the addition of 10 grams to the hammer to elicit a minimal knee-kick of the subject who was an instructor (R. K.) in the University, age 30. At 10 P.M., the time of retiring, after a day's work of teaching, it required the addition of 595 grams to the hammer to elicit a minimal knee-jerk. At 7 o'clock the following morning upon arising, it required, as on the preceding morning, the addition of ten grams to the hammer to elicit a kick.

Hence the effect of the day's work was to raise the threshold, as indicated by the necessity for increasing the strength of the stimulus to elicit a knee-kick, and of the night's rest to lower the threshold. If, at the end of the day the subject, instead of retiring, continued to work, the threshold continued to rise and if, in the morning, the subject, instead of arising, remained in bed, the threshold remained at the low morning level until toward the end of the day, when the subject became restless, the threshold began to rise.

Running a mile by one accustomed to taking exercise (physically fit) and by one unaccustomed to taking exercise, (physically unfit) each running as fast as he could, produced a greater rise in the threshold in the physically unfit than in the physically fit and the time required, during the period after the mile run, for the threshold to return to its original level was much greater for the physically unfit than for the physically fit.

Moderately low environmental temperature lowered the threshold while moderately high environmental temperature raised it. The performance of a day of hard manual labor on a farm raised the threshold more on a hot summer day than on a cold winter day.

*The validity of current evidence for existence of cerebral vasodilators.* CARL J. WIGGERS. Department of Physiology, Western Reserve University Medical School, Cleveland, O.

In 1914 I reported confirmatory observations (J. Physiol. **48:** 109, 1914) on the constricting effect of epinephrine upon vessels of the completely isolated brain, removed from the skull, and perfused with a rhythmic stream of blood-Locke's solution. In four experiments epinephrine unquestionably decreased the venous outflow and raised chiefly the diastolic perfusion pressure, *but* enlarged the pial vessels.

Such seemingly contradictory findings can be explained by assuming that unseen arterioles within the brain were actively constricted while larger surface vessels were passively distended.

The observations, though few in number, raise the question whether the larger pial vessels act as *flood gates* for the interior of the brain or as *reservoirs* accommodating the blood not allowed to pass the arteriolar stopcocks within the brain substance. In short, are observed variations in size of pial vessels, even when extracranial anastomoses are excluded and blood pressure remains constant, due to active or passive changes?

The question is pertinent because our best evidence for the existence of dilator fibers in cranial nerves rest upon observations of surface vessels. Other reactions have been presented as supplementary evidence, but changing arterial pressures or shift of blood from extra-cranial territories could not be adequately controlled in these.

Indeed it is my well considered opinion that most experimenters in drawing conclusions regarding active cerebral vasomotion have realized the importance of excluding extracranial communications, chiefly via the internal ophthalmic branch. But completely isolated brains, the vessels of which could be expected to react have not been employed in such studies between 1905 when I (Am. J. Physiol. **14:** 452, 1905) devised and reported a simple method of making such a preparation and 1936 when Bouckaert and Jourdan (Arch. internat. de pharm. et de therap. **53:** 523, 1936) achieved the second truly isolated preparation through multiple ligations. Consequently many deductions regarding active cerebral vasomotion have not been established with the same degree of surety that a physicist would require in analyses involving hydrodynamic problems of a similar nature.

*The time relationship of electrical and mechanical processes in the ventricle of the dog.* HAROLD C. WIGGERS (introduced by Magnus I Gregersen). Western Reserve University and College of Physicians and Surgeons, Columbia University, New York City.

There is apparently still some difference of opinion in regard to whether or not the electrical events precede the contraction in cardiac muscle (cf. C. J. Wiggers, Am. Heart J. **1:** 3, 1925). The evidence presented here was derived from simultaneously recorded myocardiograms, aortic pressure curves and pure monophasic action potential curves. The latter were obtained from a discrete point of surface tissue, situated near the lateral ventricular wall between two branches of the left anterior coronary artery. The onset of the monophasic curve from this locus preceded contraction in the innermost septal musculature by at least 0.01 second. Since surface tissue immediately overlying the septum is excited 0.01 to 0.017 second in advance of the remaining ventricular surface tissue, and since contraction of the innermost portion of the septum logically precedes that of its external segments by a definite though variable interval, it is deduced that the electrical event precedes contraction in surface tissue beneath the active (exploratory) electrode by 0.025 to 0.03 second.

The abrupt decline of the monophasic curve occurs prior to the attainment of maximal contraction of ventricular musculature as a whole, the latter being determined from the peak of the aortic pressure curve. Furthermore, the electrical event terminates at least 0.03 second before the onset of isometric relaxation, at which moment the surface tissue is still contracting, since it is excited rather late in the train of events. Therefore, the indications are that the electrical event is quite independent of and is not perpetuated by the mechanical response. This contention is further substantiated by the following observation: When the tissue beneath the active electrode is deprived of its blood supply by coronary ligation, it becomes ischemic, ceases to contract and is severely stretched or distended by the pressures created during the contraction of the rest of the left ventricle. Nevertheless the magnitude and form of the monophasic curves remain unaffected.

*The anti-pernicious-anemia factor is present in fetal calves' livers; or, the permeability of the placenta to the anti-pernicious-anemia factor.* H. S. WIGODSKY (by invitation), O. RICHTER (by invitation) and A. C. IVY. Department of Physiology, Northwestern University Medical School, Chicago, Ill.

An extract of fetal calves' livers was prepared by the usual method for making extracts of the liver for parenteral use in the treatment of pernicious anemia. The extract was administered parenterally to three patients who were in severe relapse with pernicious anemia. The extract proved to be very potent causing a reticulocytosis up to 49 per cent and a complete return of the hemoglobin and erythrocytes to normal. It is therefore hypothesized that the placenta is permeable to the anti-pernicious-anemia factor, which can be stored in the livers of fetal calves. Such an hypothesis is requisite to explain the presence of the factor in fetal liver, if it is assumed that Castle's theory of the origin of the active principle in liver is correct. Of course, it is remotely possible that the fetal stomach secretes the intrinsic factor and the cellular detritus of the stomach and intestine of the fetus serves as the extrinsic factor.

*Potassium changes in submaxillary glands during stimulation.* J. H. Wills, Jr. (by invitation) and W. O. Fenn. Department of Physiology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

Resting and stimulated submaxillary glands of cats, and the saliva produced during stimulation, were analysed for potassium. Two series of experiments were run; in one of these stimulation was produced electrically by a thyratron stimulator at a frequency of 15 per sec., and in the other by intravenous injection of pilocarpine hydrochloride. Average rates of secretion in these two experimental groups were not significantly different.

When the glands were stimulated electrically through the parasympathetic nerve, the stimulated glands had an average potassium content of 36.0 m.eq. per 100 g. dry wt. while the control glands contained 35.4 m.eq. per 100 g. dry wt. There was a linear relationship between the intake and output of potassium by the gland during stimulation. The glands stimulated with pilocarpine had an average potassium content of only 25.4 m.eq. per 100 g. dry wt. although the corresponding controls contained 33.4 m.eq. per 100 g. dry wt. Here there was fair agreement between the intake and output of potassium by the gland at the lower values of output, which in general were obtained with the smaller doses of pilocarpine, but as the output increased the intake fell off.

A further series of experiments was done in which the venous outflow from the submaxillary gland was isolated and the potassium content of the venous plasma obtained during stimulation compared with that of the arterial blood. On the average, whether the stimulus was electric or pilocarpine, there was a reduction of plasma potassium from 16 mg. per cent in the arterial blood to 12 mg. per cent in the venous. However, with electrical stimulation the rate of blood flow from the gland was 2–3 times that with pilocarpine stimulation. Increasing the blood potassium by intravenous injection of potassium chloride had no effect upon the secretion. This suggests that the total supply of potassium to the gland is more important in maintaining constant the potassium content of the gland than is the concentration of potassium in the plasma per se.

*The action of KCl, muscarine, pilocarpine, atropin and some other chemicals at the carotid reflex zone.* Claude V. Winder (introduced by Robert Gesell). Department of Physiology, University of Michigan, Ann Arbor.

With major branches of the carotid ligated except the lingual artery, quick intracarotid injections of 0.01 to 0.25 cc. of 0.154 M. KCl caused just discernible to moderately strong excitation of respiration, respectively. Control injections carried through the contralateral denervated occipital and internal carotid arteries, and injections on the originally innervated side after cutting only the sinus nerve, demonstrated the responses to be carotid reflexes. Larger doses (0.25 to 1.0 cc.) caused respiratory stimulation temporarily interrupted at the beginning by depression or apnea concomitant with hypotension and bradycardia (with vagi intact). The depression phase is not at this writing satisfactorily analyzed.

Under similar conditions 0.1 mgm. of "muscarine" was found to elicit moderately strong caroto-respiratory excitation. Pending exact information on the preparation furnished us, it cannot be said whether this is a contradiction of Schmidt's (1938) briefly stated opinion that the action of choline derivatives at the carotid body is exclusively "nicotinic." In this connection it is interesting that 0.1 mgm. of pilocarpine (nitrate or hydrochloride), although causing a notable hypotension upon *recirculation*, elicited none of the familiar immediate caroto-respiratory reflex. Injection of 1.0 mgm. was followed by a slow, gradual, prolonged augmentation of breathing which is to be analyzed.

A preliminary study was made of the relative potency in eliciting the caroto-respiratory reflex, of several representative chemicals. Minimal doses required to elicit a just definitely discernible excitation of breathing, when injected quickly in small quantities of isotonic fluid, were determined. The mode for nicotine alkaloid was 0.0001 mgm. With that as unity, relative doses of others were: acetyl choline hydrochloride, 10; NaCN, >10; "muscarine," >100; KCl (injected in one-tenth the fluid volume hence more quickly), <1000; trimethylammonium chloride, >1000; lactic acid, <10,000.

Atropinization of the animal, controlled by vagal cardiac action, did not eliminate responses to the above excitants. This accords with Schmidt's (1938) statement that choline and certain of its derivatives cause reflex hyperpnea after "full atropinization."

*Respiration in cat fetuses.* W. F. Windle, A. G. Steele (by invitation), C. L. Bishop (by invitation) and M. Monnier (by invitation). Department of Anatomy, Northwestern University Medical School, Chicago.

Fetuses of unanesthesized cats (carotid and basilar arteries tied an hour or more previously) examined in utero and in the early minutes after Caesarian section with placenta intact were considered to show normal physiologic behavior providing maternal respiration and body temperature were little affected at the time of observing the fetuses. These normal fetuses were apneic during the greater part of the 64 day gestation period. Conditions lead to asphyxia in mother and fetus inaugurated rhythms of delicate, quick fetal respiratory movements between about 31 and 53 days. Carbon dioxide (8 to 10 per cent) in oxygen, breathed by the mother, initiated similar movements in normally apneic fetuses. However, asphyxia and carbon dioxide excess readily depressed the rate of these movements when allowed to over-act and led to gasping and sustained contractions of trunk and limb (principally extensor) muscles.

In contrast to these results, larger fetuses of 62, 63 and, with labor inhibited by producing fresh corpora lutea hormonally, 65 days executed short interrupted respiratory rhythms normally in utero. Anoxemia and carbon dioxide served only to depress them. When fetuses near term (62 days) were found to be apneic due to excessive anoxemia, respirations could not be started by carbon dioxide but forcing the mother to breathe pure oxygen brought them about promptly.

Uterine contractions attained sufficient strength in one experiment to start downward progression of the undelivered (65 day) fetus which was the nearest to the vagina. With each uterine contraction, reddening of the umbilical vein in the delivered fetuses, whose placentae were still intact, was observed and fetal respirations, which had become depressed, were stimulated at this time. Analyses of the umbilical vein blood showed a higher oxygen content during the contractions than during the quiescent periods.

We conclude that cat fetuses are apneic in utero so long as there an is adequate exchange of gases through the placenta. Fetal respirations start when this mechanism becomes impaired experimentally or physiologically. During labor a means seems to be provided for periodically relieving a tendency toward asphyxia.

*Changes in fat metabolism produced by early liver damage.* IRWIN C. WINTER (introduced by L. A. Crandall, Jr.). Department of Physiology and Pharmacology, Baylor University College of Medicine, Dallas, Texas.

The fasting lipid values of dogs' plasma were followed before and for two weeks following the production of liver injury by the administration of carbon tetrachloride (1 ml. per 5 kgm. body weight per day). Fecal "fat" excretion was also determined, and the daily food intake observed.

The amount of total fatty acids in the plasma at the end of six days treatment averaged 287 mgm. per cent (original 349 mgm. per cent), and the iodine number was 106 (original 113). The fecal "fat" increased to 1.78 grams per day from an original 0.64 gram. The food intake had decreased 50 per cent, and the weight loss of the animals was 10 per cent (average). At the end of two weeks treatment the total plasma fatty acid value was 267 mgm. per cent (iodine number—60), the fecal "fat" excretion 1.27 grams per day, the food intake was only 23 per cent of the original amount, and the weight loss was 21 per cent. While the iodine number of the phospholipid fatty acids decreased to 100 (from 157) at the end of two weeks treatment, there was no change in relative amount (per cent of total fatty acids). The liquid/solid acid ratio of this fraction remained constant. The iodine number of the fecal "fat" did not change appreciably during the entire period of observation.

*Functional connections established by spinal nerves regenerating into preganglionic sympathetic nerve trunks.* H. G. WOLFF, KENDRICK HARE (by invitation) and MCKEEN CATTELL. Departments of Physiology and Pharmacology, Cornell University Medical College, and the Department of Medicine, New York Hospital, New York City.

The central end of the phrenic fibers contributed by the fourth and fifth cervical nerves was sutured to the distal portion of the cut cervical sympathetic trunk on the left side in 5 cats. The anastomosis was made 3–4

cm. below the superior cervical sympathetic ganglion. After a period of from 7 to 11 months to permit regeneration, the function of the newly established pathways was tested. Respiratory hippus was observed in two of the five animals. Further study of functional connections was carried out under "dial" anaesthesia. The response of the nictitating membrane was photographically recorded during stimulation of the phrenic nerve proximal to the anastomosis. In three animals such phrenic nerve stimulation produced contraction of the nictitating membrane. In two of the three animals, nicotine was applied locally to the superior cervical ganglion. In one, complete block was obtained. In the other it was impossible to abolish the effect of the phrenic nerve stimulation, although similar nicotinization of the intact side produced complete block.

At the termination of the experiment, portions of the left phrenic nerve proximal and distal to the anastomosis were stained with osmic acid and by Bodian's silver method. Similar preparations were made of the right vago-sympathetic trunk. Both superior cervical sympathetic ganglia were cut in serial sections and stained with silver. Examination of this material revealed considerable regeneration of nerve fibers from the proximal part of the sympathetic trunk. The accurate identification of phrenic fibers (12–17$\mu$) was also possible and showed their growth up the sympathetic trunk and into the ganglion where they branched repeatedly in the vicinity of the post-ganglionic neurones. Synaptic relations of these phrenic fibers with the post-ganglionic sympathetic neurones were seen in each of the five animals. Some of the large phrenic fibers passed through the ganglion and out via the post-ganglionic paths.

*Studies on the effect of the digitalis glucosides on potassium loss from the heart of the heart lung preparation.* E. H. Wood and G. K. Moe (introduced by M. B. Visscher). Department of Physiology, University of Minnesota, Minneapolis.

A number of previous observations have called attention to the importance of potassium concentration changes in muscle activity and in the pharmacological action of digitalis. We have studied these questions in 36 heart lung preparations and in a number of control experiments. Our results are summarized in the following table where the increased plasma potassium is expressed as milligram of potassium gained by the plasma per hour per gram of heart.

| | |
|---|---|
| Heart lung Prep. receiving no drug (6 exp.) | 0.056 mgm. |
| Heart lung Prep. receiving therapeutic doses (9 exp.) | 0.103 mgm. |
| Heart lung Prep. receiving toxic doses (15 exp.) | 0.329 mgm. |
| Stirred blood and perfused lung Rec. toxic doses (3 exp.) | 0.052 mgm. |
| Stirred blood and perfused lung Rec. no drug (4 exp.) | 0.032 mgm. |

The hearts from eighteen experiments have been analyzed for potassium and water content. Only one of these hearts received no drug. Five hearts from dogs killed by hemorrhage have also been analyzed. The results are summarised below:

| | *Per cent water* | *K mgm. per cent wet weight* | *K mgm./gm. dry weight* |
|---|---|---|---|
| Bleeder hearts (5) | 76 | 312 (275–336) | 13.0 |
| Heart lung hearts (16) | 77 | 241 (175–280) | 10.2 |
| Heart lung heart (no drug) (1) | 77 | 286 | 12.6 |

It is obvious that although there is a small spontaneous increase in plasmapotassium over time and a small decrease in heart muscle potassium, the rate of change is greatly increased by toxic doses of digitalis.

*The effect of anaphylactic shock on gastric secretion.* PARKE H. WOODARD and N. P. SHERWOOD (introduced by O. O. Stoland). University of Kansas, Lawrence.

Lewis Dale and others have suggested that the symptoms of anaphylactic shock are due to the release of histamine or histamine-like substance within the body of a sensitized animal upon initiation of shock. It was suggested that the well known effect of histamine on gastric secretion might be used to test this hypothesis. At first Pavlov pouch dogs were sensitized to horse serum by the Manwaring technic and the gastric secretion studied before and after induction of shock, but the blood pressure drop was so great as to, of itself, interefere with stomach secretion. Accordingly, the pouch dogs were used as test animals and shock induced in other sensitized animals from which blood was drawn and injected subcutaneously into the pouch dogs.

The general procedure was as follows: First, the pouch dogs were tested as to their response to subcutaneous injection of histamine. Next, blood was withdrawn from the sensitized dogs before shock was induced and injected subcutaneously into the pouch dogs—coagulation being prevented by heparin. Finally, blood was withdrawn after the induction of shock and likewise injected subcutaneously into the pouch dog. The gastric response in the pouch dog was studied in serial ten-minute periods as to volume of secretion, free acid and total acid. Studies have so far been made on five dogs in shock using two Pavlov pouch animals.

In all these the shock blood tended to cause an increase in both volume and acidity of the gastric juice with no increase produced by the blood previous to shock. The reaction is delayed somewhat and more prolonged than with the injection of a single dose of histamine in salt solution, and there is some evidence that the substance which stimulated gastric secretion is produced late in shock which may indicate that it is secondary. Further work is being done on these points.

*Pulmonary arterial, venous, and differential pressures in the unanesthetized dog.* R. A. WOODBURY, W. F. HAMILTON and ELKIN VOGT (by invitation). Department of Physiology and Pharmacology, University of Georgia School of Medicine, Augusta.

Following a modification of the London technique silvered flanges were sewed on the pulmonary artery and vein. In the two stage operation these flanges were allowed to become well fixed in scar tissue. In the one stage operation the next step followed immediately. Through separate stab wounds were inserted silvered cannulae shaped like hollow nails with heads large enough to keep the cannulae from slipping back between the ribs. Threads in the stems of these cannulae were screwed tightly into the flanges on the vessels. The skin was sewed over the heads of the cannulae and the chest closed.

After the animal recovered, the skin over the heads of the cannulae was infiltrated with procaine and pressures in the vein and artery were recorded with hypodermic manometers. Two 3 inch hypodermic needles, connected to the manometers, were inserted into the artery or vein.

The manometers were set up to record (1) pulmonary arterial pressure, (2) pulmonary venous pressure, and (3) a differential record of arterial minus venous pressure in which the venous pressure was instrumentally subtracted from the arterial pressure, as described in these proceedings last year.

The arterial pressure curves show an anacrotic halt, a high systolic peak and a low flat diastolic curve. In the vein the A, C, V waves are clear. Average pressure values were: arterial 40/10, venous 2–12 and differential 30/4 mm. Hg (6 unanesthetized dogs). Marked simultaneous respiratory fluctuations were seen in the pulmonary arterial and venous pressures. They were the direct propagation of intrathoracic pressure changes and did not appear in the differential pressure record which shows the head of pressure forcing blood through the lungs. A large increase in both arterial and venous pressure occurred in response to decompensatory doses of epinephrine. No rise in the differential pressure was seen. Drugs which slowed the heart widened the pulse pressure (epinephrine 4 to 10 y/K.). Drugs which accelerated the heart narrowed the pulse pressure (atropine, mecholyl and amyl nitrite). The above drugs and morphine produced no definite change in mean pulmonary arterial or venous pressures, nor did they affect the differential pressures. It is concluded that the capacious pulmonary bed minimized the effects of volume flow changes or possible vasomotor reactions upon the pulmonary pressures.

Aid in pursuance of this investigation is acknowledged from the American Medical Association.

*Representation in the motor cortex of flexor and extensor muscles of the leg.*

Clinton N. Woolsey (introduced by Philip Bard). Department of Physiology, Johns Hopkins Medical School, Baltimore, Md.

Action potential study (Marshall, Woolsey, Bard, 1937) of cortical representation of tactile sensibility in the monkey has revealed for all portions of the body caudal to the arms a cortical sequence which accurately reflects the metameric origin of the dermatomes. In the case of the leg the preaxial surface is represented, from hip to hallux, on the dorsal aspect of the hemisphere; the postaxial surface, including the remaining digits, is represented, from toes to hip, on the medial aspect of the hemisphere above the centers for perineum and tail.

Is there a similar arrangement in the motor cortex? This has been tested in a series of monkeys with the interesting result that those muscles derived from the ventral muscle sheet of the limb bud (primitive flexors) are represented on the dorsal aspect of the hemisphere, while those derived from the dorsal muscle sheet (primitive extensors) are represented chiefly on the medial aspect. On stimulating successive points extending from the trunk area of the precentral gyrus to the tail area at the sulcus cinguli the movements obtained are as follows (omitting rotation, abduction and adduction): ventrolateral flexion of trunk, flexion (protraction) of hip, flexion of knee, plantar flexion of foot, inversion of foot, flexion of toes, extension of toes, extension (dorsiflexion) and eversion of foot, extension of knee, extension (retraction) of hip, constriction of anus, elevation and contralateral deviation of base of tail, lateral deviation and flexion of tip of tail. Adjacent motor and sensory points show a nice correspondence as regards the limb segment represented.

On the postcentral gyrus skin areas are represented by strips of cortex extending diagonally in a posterolateral direction from the central fissure.

Motor representation also shows this diagonal pattern, but the motor diagonals take an anterolateral direction. Therefore, corresponding sensory and motor diagonals together form a herring-bone design. Diagonals from the medial surface pass over the rim of the hemisphere onto the dorsal surface.

*On the central action of acetylcholine.* JOSEPH WORZNIAK (by invitation) and ROBERT GESELL. Department of Physiology, University of Michigan, Ann Arbor.

Breathing was commonly increased by the action of acetylcholine through the floor of the fourth ventricle, by intracarotid injection beyond the carotid body, by intravenous injection, and at the carotid body. The effects varied in somewhat characteristic ways, depending upon the mode of administration. Intravenous injections of eserine commonly produced a late increase in breathing. Eserine injections have sometimes been followed by increased effects of subsequent administrations of acetylcholine on the floor of the fourth ventricle and intra-arterial injection beyond the carotid body thus tentatively suggesting a central potentiating action.

*Adrenalin hypersensitivity in denervated intestinal fistulae.* W. B. YOUMANS (by invitation) and W. J. MEEK. University of Wisconsin, Madison.

There is much evidence that smooth muscle which is stimulated by adrenalin shows increased sensitivity to adrenalin after degeneration of its sympathetic nerves. However, concerning sensitization by denervation of smooth muscle inhibited by adrenalin, reports have been few and contradictory (Luco, 1937).

In three dogs, each having both an innervated and a denervated jejunal fistula made from adjacent segments, records of the motility of the fistulae were taken by the balloon method during the injection of adrenalin at various constant rates. The animals were not fastened or medicated.

Injection rates which produced initial complete inhibition in the denervated fistulae were 0.14, 0.20, and 0.28 gamma/kilo/minute. These injection rates were doubled and, in one animal, trebled without producing definite inhibition in the innervated fistulae. Each increase in the rate of injection resulted in a proportionately longer duration of complete inhibition in the denervated fistulae, but escape from the inhibition occurred as the constant rate of injection was maintained. Associated with the inhibition of rhythmic contractions there was decreased tonus.

Sudden loud noise caused the appearance of a short period of complete inhibition in the denervated fistulae of the same dogs with only slight or no effects on the innervated fistulae. This effect was not duplicated in an animal having no adrenal medullary tissue.

Denervation of the dog intestine makes it one of the most sensitive of indicators for the autoassay of adrenalin and renders it much less resistant than normal to the motility- and tonus-inhibiting effect of adrenalin and possibly of adrenalin-like neurohormones.

*Modification of radiosensitivity by intracellular acidity.* R. E. ZIRKLE (introduced by D. W. Bronk). Johnson Foundation, University of Pennsylvania, Philadelphia.

In earlier work it was found that the x-ray sensitivity of a certain type

of cell (fern spore) was modified by penetrating acids and bases. When radiosensitivity was plotted against the concentration of acid or base in the culture medium during irradiation, the resulting curve exhibited three maxima and two intervening minima, which suggested that the radiation might be acting on the cell through radiochemical alterations in three different amphoteric substances.

This work has been extended by making use of the facts that radiochemical reactions often deviate from the Bunsen-Roscoe law and that the amount of deviation is usually characteristic for a given reaction. The material was irradiated in two series at two levels of intensity (with correspondingly different durations). The doses used in the two series were adjusted to give equal effects upon the samples not treated with acid or base. In the treated samples it was then found that the dosages of the two series were not equally effective. With the values of intracellular acidity corresponding to two of the maxima in the radiosensitivity curve, high intensity was more effective than low; the opposite was true at the third maximum.

Results of this nature could be expected if three radiochemical reactions were involved, each having its characteristic time-intensity relations.

*Studies on the source of increased blood potassium after nerve excitation.* R. L. ZWEMER and F. H. PIKE. Departments of Anatomy and Physiology, College of Physicians and Surgeons, Columbia University, New York City.

Generalized nerve excitation by camphor-monobromide injection is followed by increased serum potassium. Increases also occur after temporary cerebral anemia with the resultant sympathetic discharge and after electrical stimulation of intact splanchnic nerves (Zwemer and Pike, Annals New York Acad. Sci., **37:** 1938). Elevation of cerebro-spinal fluid potassium, and evidence from the literature that stimulated nerves release potassium, suggested that nervous tissue might be responsible for part of the rise. The contribution from nervous tissue was however comparatively small, since samples of venous blood coming from brain, liver, skeletal muscle and intestine showed that after general nerve excitation portal blood contained the greatest increment of potassium. The lower hepatic vein values suggest that the liver may remove potassium from portal blood, although dilution by arterial blood must be considered. Repeated electrical stimulation of vagus, sacral autonomic, sciatic and afferent splanchnic nerves failed to raise blood potassium to the same extent as could be attained by intact splanchnic or by efferent splanchnic stimulation. These experiments suggest the intestine as the source of the increased blood potassium. The normal constancy of serum potassium attest to the efficiency of the K regulating mechanism of the body, and the lack of change in many of our experiments meets the criticism that the potassium changes might be the result of the operative procedures rather than the nerve stimulation. In work on shock (Zwemer and Scudder, Am. J. Physiol. **119:** 427, 1937) it was found that considerable tissue damage was necessary in order to effect an alteration in the blood potassium level. Commercial adrenaline given in large quantities did not raise blood potassium to the same extent as repeated efferent splanchnic stimulation, although it was injected into the femoral vein and into the superior mesenteric artery.

# EXPERIMENTAL RENAL HYPERTENSION AND ADRENALECTOMY[1]

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It is well known that constriction of the blood vessels of the kidneys results in arterial hypertension (1, 2, 3), but the mechanism has not been fully elaborated. It has been demonstrated that the nerves of the kidney are not concerned since hypertension still develops when the arteries of denervated kidneys are constricted (4, 5) or even after constriction of the artery of a transplanted kidney (6, 7). Therefore it is probable that the mechanism is a humoral one. An excellent review of the literature of this problem is to be found in a recent paper by Goldblatt (8). In this same paper some preliminary experiments are reported, which suggest that the adrenal cortex may be involved. Goldblatt finds that adrenalectomized dogs who receive either supportive treatment alone (sodium chloride and sodium bicarbonate or sodium citrate) or no treatment at all do not develop or maintain this type of hypertension; but that, if both supportive treatment and adrenal cortex extract are given or if a remnant of cortical tissue is left, the animals may develop a significant elevation of blood pressure. He tentatively suggests that when the renal arteries are constricted a substance is liberated from the ischemic kidney and that the adrenal cortical hormone may play a part in conjunction with this hypothetical substance in elevating the blood pressure. Blalock and Levy (7) have recently reported somewhat similar results, namely, that adrenalectomy, followed by administration of sodium chloride, sodium citrate, glucose, and a low protein diet, quickly abolishes experimental renal hypertension. Goldblatt (3) has also shown that the adrenal medulla is not concerned in this type of high blood pressure.

The present study, which was begun about a year ago, is concerned

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The work has also been aided by the services of B. A. Duffy, a worker assigned by the Works Progress Administration.

Part of the adrenal cortex extract used was furnished through the courtesy of Parke Davis and Company.

with the rôle of the adrenal cortex in experimental renal hypertension, with the effect of the removal of the adrenal glands on such hypertension. The fact that the adrenal cortex is important in maintaining the normal level of blood pressure constitutes a complicating factor. In cortical insufficiency there occurs, among other things, an increased potassium in the blood plasma, a loss of sodium, lowering of the plasma volume, and a drop of arterial blood pressure. The failure of development of renal hypertension or reduction of an already existing hypertension in animals after adrenalectomy may simply be an expression of the changes occurring in cortical insufficiency; and, in order to demonstrate a more specific rôle of the cortical hormone, the disturbance in plasma volume and other changes responsible for the lowering of blood pressure ordinarily occurring as a secondary effect of cortical insufficiency must be avoided. We have attempted to accomplish this by the use of a diet high in sodium and low in potassium supplemented by the use of adrenal cortex extract only at critical times. The importance of the low potassium diet has been pointed out by Allers and Kendall (9).

Three types of experiments have been performed on dogs. In the first set (five dogs) hypertension was produced by constriction of the renal arteries, and the adrenal glands were removed subsequently. In the second type (two dogs) adrenalectomy was first performed, and, following this, constriction of the renal arteries was carried out. The last set consists of one control in which simple adrenalectomy was carried out.

METHODS. The blood pressure was measured by means of a carotid loop using the Van Leersum method (10). The normal level was determined by tri-weekly measurements extending usually over a month. The renal arteries were constricted by a method described previously by one of us (5). In all cases except one both arteries were constricted. Adrenalectomy was carried out in two stages; the right gland was first removed, and, after healing had occurred, the left gland was excised. In some animals at the time of removal of one or other of the adrenal glands, the corresponding renal artery was reconstricted. Following the removal of the second adrenal gland, the dogs were tided over the effects of the operation with an ordinary diet (usually "Pard," a canned animal food) and subcutaneous injections of adrenal cortex extract (usually Wilson's), and were then maintained on a high sodium intake and a diet low in potassium as described by Allers and Kendall (9). When the dogs refused to eat the special diet, they were given small doses of adrenal cortex extract and meat in the diet for one or two days. In the surgery on already adrenalectomized animals adrenal cortex extract and an ordinary diet were again given for a period immediately after the operation, following which the high sodium, low potassium régime was resumed. The diet had the following percentage composition: casein, 23.7; sucrose,

42.6; lard, 17.5; butter, 6.7; agar-agar, 2.3; dried yeast, 1.9; salt mixture, 0.7; sodium chloride, 2.5; sodium citrate, 2.1. An amount of the above diet equivalent to 80 Calories per kilogram of body weight was given daily. The salt mixture used in this diet had the following percentage composition: sodium phosphate (dibasic), 36.7; calcium diphosphate, 15.6; ferric citrate, 9.6; sodium iodide, 1.1; magnesium citrate, 37. Sodium chloride, to make an 0.8 per cent solution, was added to the drinking water. In general the adrenalectomized animals got along nicely on the above régime, were active and alert, and did not lose weight.

Blood urea nitrogen, plasma potassium, and plasma sodium were determined at intervals in order to check the condition of the animals. Urea nitrogen was determined by the urease method of Van Slyke and Cullen as described by Peters and Van Slyke (11) with aeration and titration of the ammonia formed; potassium by a colorimetric method based on that of Breh and Gaebler (12); and sodium by the gravimetric method of Barber and Kolthoff (13). All analyses were done in duplicate or triplicate.

The completeness of the adrenalectomy was confirmed at the end of the experiment by discontinuing the high sodium, low potassium régime. In all, except in two cases where the animals died otherwise, the dogs died in two to four days with elevated plasma potassium, lowered plasma sodium, and hypotension.

Results. The effect of adrenalectomy on a previously established hypertension (table 1 and fig. 1) was a gradual reduction of the elevated pressure. In two experiments even the removal of one adrenal resulted in a fall. (The other experiments do not permit any statements as to the effect of the removal of one adrenal gland. In dogs 2 and 4, at the time that the first adrenal was removed, the corresponding renal artery was reconstricted. In dog 5 only one renal artery was constricted, and blood pressure eventually tends to return toward the original level in unilateral constrictions.) The removal of the second adrenal resulted in a progressive fall of blood pressure even in cases in which the corresponding renal artery was reconstricted at the time. In no case, however, did the blood pressure reach its normal level in the time allowed in these experiments. The plasma sodium and plasma potassium showed no significant alteration on the development of hypertension. After the removal of both adrenal glands, in spite of the high sodium, low potassium régime, the plasma sodium fell, and there was a slight rise in the potassium in two of three experiments. The blood urea nitrogen was within normal limits.

When the renal arteries of previously adrenalectomized animals were constricted, hypertension developed. During the period immediately following the constriction, when the blood pressure was rising, the dogs were receiving daily doses of adrenal cortex extract in order to tide them over the effects of the operation. The elevation of blood pressure was

not well maintained; there was a tendency for the pressure to return gradually toward the original level, but not reaching it in the duration of these experiments even in periods when no adrenal cortex extract was given. Two experiments of this type were performed; the data are summarized in table 2 and figure 2. Again the sodium fell after adrenalectomy,

TABLE 1

*Adrenalectomy following renal artery constriction*

| | DOG 1 | DOG 2 | DOG 3 | DOG 4 | DOG 5* |
|---|---|---|---|---|---|
| Average normal systolic pressure (mm. Hg) | 122 | 108 | 108 | 135 | 117 |
| Average pressure after development of hypertension | 169 | † | 186 | ‡ | 186 |
| Length of this control period of hypertension (days) | 15 | 18 | 28 | 75 | 19 |
| Pressure after removal of 1st adrenal (average of 10 days preceding removal of 2nd adrenal) | 153 | 157 | 152 | 180 | 163 |
| Number of days between removal of 1st and 2nd adrenals | 69 | 19 | 54 | 11 | 17 |
| Pressure after removal of 2nd adrenal (average of 10 days preceding end of experiment) | 136§ | 131 | 138 | 177 | 126 |
| Days elapsing between removal of 2nd adrenal and end of experiment | 42 | 23 | 13 | 5 | 40 |
| Normal plasma sodium (mgm. per 100 cc.) | 351 | | 340 | | 327 |
| Normal plasma potassium (mgm. per 100 cc.) | 18 | | 18 | | 15 |
| Sodium after development of hypertension | | | 332 | | 334 |
| Potassium after development of hypertension | | | 19 | | 18 |
| Sodium after removal of 1st adrenal (shortly before removal of 2nd adrenal) | 301 | | 339 | | |
| Potassium after removal of 1st adrenal (shortly before removal of 2nd adrenal) | 19 | | 18 | | |
| Sodium after removal of 2nd adrenal (near end of experiment) | 277 | 299 | 326 | | 306 |
| Potassium after removal of 2nd adrenal (near end of experiment) | 24 | 25 | 16 | | 24 |
| Blood urea nitrogen after removal of 2nd adrenal (near end of experiment—mgm. per 100 cc.) | 13 | 14 | 16 | | 9 |
| Number of days of adrenal cortex extract therapy immediately following removal of 2nd adrenal | 4 | 6 | 6 | 5 | 7 |
| Subsequent number of days on which adrenal cortex extract was given | 5 | 0 | 0 | | 3 |
| Manner of death | Special diet stopped | Special diet stopped | Adrenal crisis | Hemorrhage | Killed |

* Only the right renal artery was constricted in this experiment.

† The right renal artery was first constricted; next the left adrenal was removed and the left renal artery constricted at the same time; finally the right renal artery was reconstricted.

‡ The renal arteries were constricted several times. Finally the first (left) adrenal was removed, and the left renal artery was reconstricted.

§ The left renal artery was reconstricted at the time of removal of the left adrenal gland.

and the plasma potassium became somewhat elevated. In one of the dogs the urea nitrogen remained within normal limits, while in the other it increased slightly near the end of the experiment.

In the two experiments just described above it will be noted that there was a slight rise of blood pressure following the removal of the second

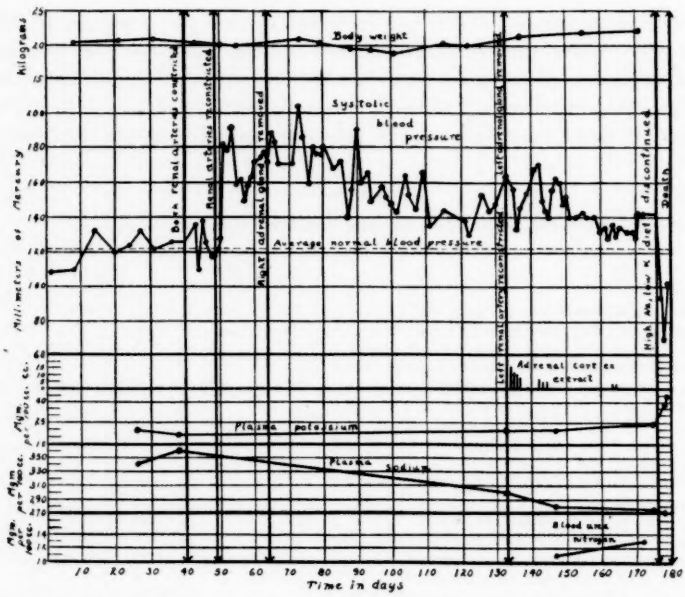


Fig. 1. Dog. 1. Hypertension was first produced by constriction of both renal arteries; following this the adrenal glands were removed. The left renal artery was reconstricted at the time that the left adrenal gland was removed.

TABLE 2

*Dog 6. Renal artery constriction following adrenalectomy*

| | PERIODS | AVERAGE SYSTOLIC BLOOD PRESSURE | PLASMA Na | PLASMA K | BLOOD UREA N | ADRENAL CORTEX EXTRACT GIVEN |
|---|---|---|---|---|---|---|
| | *days* | *mm. Hg* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *mgm. per 100 cc.* | *days* |
| Normal | 1–50 | 115 | 335 | 19 | | 0 |
| After removal of right adrenal | 51–69 | 117 | 335 | 18 | 15 | 0 |
| After removal of left adrenal | 70–82 | 135 | | | | 5 |
| | 83–90 | 158 | | | | 8 |
| | 91–95 | 196 | | | | 5 |
| After renal artery constriction | 96–100 | 169 | 307 | 14 | 13 | 3 |
| | 101–105 | 173 | | | | 2 |
| | 106–110 | 154 | | | | 1 |
| | 111–115 | 142 | 259 | 25 | 14 | 0 |

adrenal before the renal arteries were constricted. This fact led us to perform a control experiment in order to determine over a more extended period the effects of adrenalectomy and the treatment used subsequently

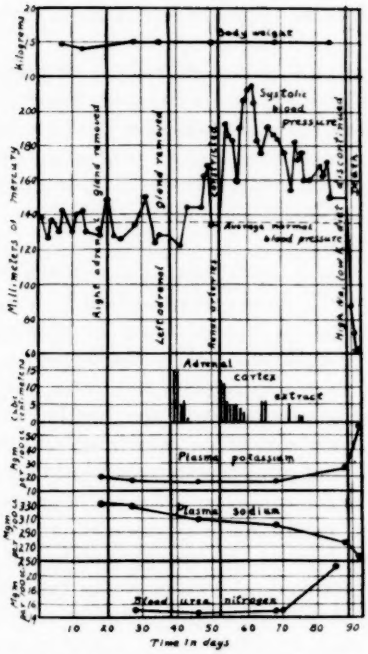


Fig. 2. Dog 7. Both adrenal glands were first removed. Following this both renal arteries were constricted.

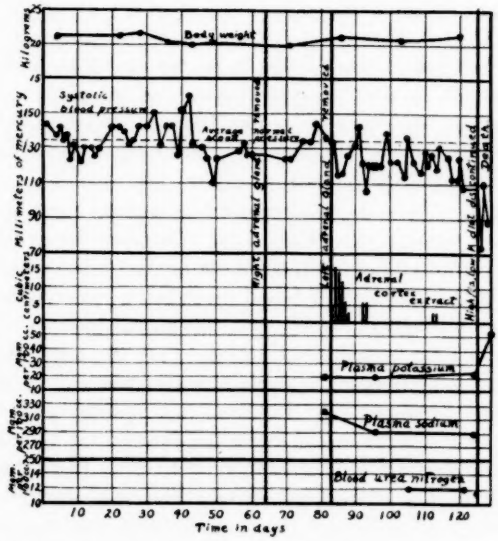


Fig. 3. Dog 8. In this animal the only procedure was the removal of the adrenal glands.

(i.e., the high sodium, low potassium régime, supplemented with adrenal cortex extract). In this experiment the tendency of the blood pressure over a period of about 40 days following the adrenalectomy was to fall slightly below the normal level (fig. 3). As in the previous experiments, despite the high sodium, low potassium diet, the plasma sodium tended to fall, and there was a slight rise of plasma potassium. The urea nitrogen was within normal limits.

Discussion. It may be concluded that adrenalectomy when followed by the measures used in these experiments (i.e., a high sodium, low potassium diet supplanted at critical times by administration of adrenal cortex extract) does gradually reduce the hypertension resulting from renal ischemia. In these experiments the blood pressure did not return entirely to the average normal level, and this held true even in periods when no cortical extract was given, which were in some cases about 15 days. It is possible that, had the animals been followed for longer periods, the blood pressure might have fallen to the normal level or below it. We do not, however, regard these experiments as an indication that the adrenal cortex is involved in any specific manner in the etiology of experimental renal hypertension. Undoubtedly the adrenal cortex is important in the maintenance of blood pressure in the case of hypertension, just as it is in the animal with a normal blood pressure. The cortex is important, for example, in maintaining the sodium and potassium balance in the body, the plasma volume, and the blood pressure; but these experiments do not indicate that it is involved in renal hypertension in any manner other than in this sense. The downward tendency of the blood pressure after adrenalectomy in the hypertensive dogs in the above experiments may well be due to an inadequacy of the after treatment used to maintain proper salt balance. The blood chemistry did not remain entirely normal; in spite of the high sodium and low potassium diet there was eventually some fall in plasma sodium and usually a slight rise in plasma potassium. Also adrenalectomized animals tend to develop an anemia (Pfiffner, Swingle, and Vars, 14). This may be another factor in determining the gradual fall of blood pressure after adrenalectomy. Until it can be shown that the fall of blood pressure in experimental renal hypertension after adrenalectomy is not due to inadequacies in the after treatment, it must be regarded as unlikely that the adrenal cortex is involved in any specific manner in such hypertension. It is interesting to note that Jeffers, Lindauer, and Lukens (15), dealing with hypertension produced by intracisternal kaolin injection, found that the elevated pressure was sustained after adrenalectomy so long as their dogs were well maintained by the use of cortical extract and salt; but that, when an attempt was made to maintain these animals by the use of salt alone,

signs of cortical insufficiency and a fall of blood pressure occurred. The fact that a hypertension of different etiology in this instance reacts similarly to adrenalectomy also argues against a specific rôle of the adrenal cortex in experimental renal hypertension.

## SUMMARY AND CONCLUSIONS

Dogs with an elevated blood pressure resulting from constriction of the renal arteries were adrenalectomized. Also the renal arteries of dogs, previously adrenalectomized, were constricted. The adrenalectomized animals were treated at critical periods with adrenal cortex extract (given subcutaneously) and otherwise by a diet containing relatively small amounts of potassium and large amounts of sodium chloride and sodium citrate.

Adrenalectomy, followed by the treatment summarized above, results in a gradual reduction of an already established hypertension.

When the renal arteries of dogs previously adrenalectomized and maintained as described above are constricted, hypertension develops. The elevation of blood pressure is poorly maintained, however, and a downward tendency soon manifests itself.

In the duration of these experiments the blood pressure of adrenalectomized dogs with constricted renal arteries, while it progressively declined, did not fall in any case to the average normal level even in periods when no cortical extract was given.

The adrenalectomized animals eventually showed a fall in plasma sodium, and most of them showed some rise of plasma potassium.

It is unlikely that the adrenal cortex is involved specifically in the etiology of experimental renal hypertension other than in the sense that the cortex is important in the maintenance of blood pressure in normal as well as in hypertensive states. The downward tendency of the blood pressure curve in the above experiments may be due to the fact that the supportive therapy was not entirely adequate.

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# NITROGEN EXCRETION ON A NITROGEN-FREE DIET

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A number of workers have commented on the shifting level of endogenous nitrogen excretion on N-free diets. In studying the biological value of proteins with the use of rats it is common practice to begin the work by feeding a N-free diet. The test protein or proteins to be assayed are then fed in sequence, and finally the N-free diet is again fed. In such studies the endogenous excretion of N as measured on the first N-free period is frequently different from the value found in the last period. In computing the biological value of the protein from the data so obtained a choice can be made between the two values found for endogenous N excretion, or an average of the two can be used. The figure used for the endogenous nitrogen coefficient will directly affect the biological value of the protein.

The possibility remains that if enough time were allowed for the animals to reach a minimum level of N excretion nearly identical figures would be obtained for endogenous N excretion on two different periods of measurement. There is, however, no agreement in regard to the time required by rats to reach a minimum level of N excretion. The work of Ashworth and Brody (2) indicates that about 3 to 8 days or more are required. Mitchell (3) and Mitchell and Carman (5) believe the rat rapidly reaches the endogenous level. Mitchell, Burroughs and Beadles (4) have shown that the assumptions underlying the calculation of biological values for proteins are, on the whole, correct and possess absolute as well as relative significance. This does not, however, clarify our fundamental understanding of the adjustment which the organism makes when fed a N-free diet. As a part of the work in that direction the experiments described below were conducted.

Rats were fed a N-free diet much longer than has generally been done in the past, namely, 60 days. The data on urine N excretion and body weight decline were reduced to general equations by the use of statistical methods. It was found that the decline in urine N excretion is gradual and follows a log-log relationship while the decline in body weight follows

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an arith-log relationship. It was further found that the urine N coefficient declines over a period of about 50 days and thereafter tends to increase slightly.

The rats used weighed on the average 70.6 grams at the beginning of the experiment and were 35 day old Wistar strain animals. The composition of the N-free diet was the same as the one used previously (7). A yeast concentrate supplement was fed daily and contained 4.3 mgm. N. The technique of Ashworth and Brody (2) was used for the collection and analysis of excreta, except that carmine was used for marking fecal excretions. Urine was collected and analyzed every 2 days for the first 20 days, and every 5 days thereafter. With but few exceptions the animals were weighed daily.

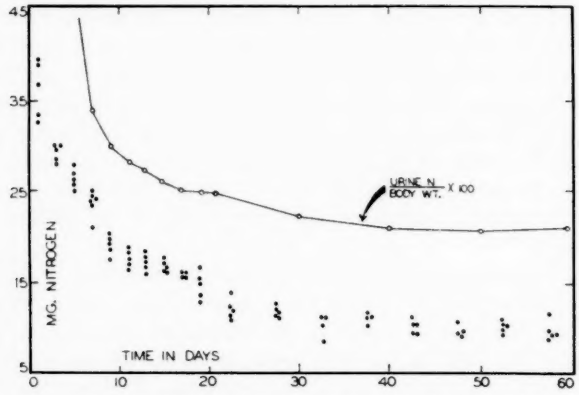


Chart 1. The urine nitrogen excretion of 5 rats is shown. The urine N/body weight ratios were computed from the general regression equations given and discussed in the text.

On chart 1 the values for total urine N excretion have been plotted. The equation which best describes this orderly decline in excretion is a log-log relationship. In computing the constants for the equation (all animals included) the first 4 days were omitted for obvious reasons, and the following was obtained: $Y = 1.703 - 0.423X$, where $Y$ represents urine N excretion in milligrams and $X$ represents time in days. The correlation for this equation was high; namely $r = -0.979 \pm 0.007$ (chart 2).

The decline in body weight was studied in a similar manner. The figures for the first 5 days were omitted, because they are directly influenced by the stock diet from which the animals were taken, and deviate appreciably from any general regression equation one attempts to apply. The decline in body weight follows an arith-log relationship and can be summarized in the following equation: $\log Y = 1.84 - 0.0035X$. In

this instance $Y$ represents body weight in grams and $X$ the time in days. The correlation coefficient for the equation was $r = -0.98 \pm 0.006$. This correlation establishes with a high degree of certainty that the change is an arith-log regression. This is in accord with the work of Ashworth (1) who obtained a straight line when the body weight was plotted on semi-log paper.

With these general equations we can next compute the average body weight and the average quantity of urine N excretion at any given time on the diet, and from the figures so obtained compute the urine N coefficient. When this is done (chart 1) it is found that the coefficient

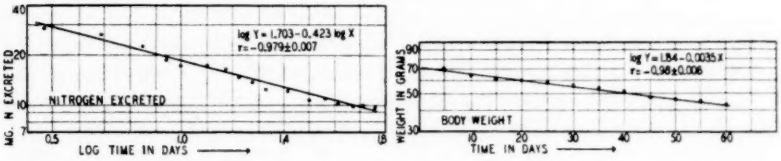
log Y = 1.703 - 0.423 log X
r = -0.979 ± 0.007
NITROGEN EXCRETED
MG. N EXCRETED
LOG TIME IN DAYS
log Y = 1.84 - 0.0035 X
r = -0.98 ± 0.006
BODY WEIGHT
WEIGHT IN GRAMS
TIME IN DAYS

Chart 2. The data for body weight and urine nitrogen excretion are plotted on logarithmic scales.

TABLE 1

*Milligrams of N excreted daily in the feces*

| RAT NUMBER | TIME ON N-FREE DIET | | | |
|---|---|---|---|---|
| | 1-8 days | 9-20 days | 21-49 days | 50-67 days |
| R-1 | 5.98 (1.06)* | 4.73 (0.99) | 3.47 (0.79) | 3.37 (0.76) |
| R-2 | 6.58 (0.92) | 5.06 (1.05) | 3.73 (0.83) | 3.70 (0.99) |
| R-3 | 7.99 (1.21) | 4.87 (1.08) | 3.95 (0.84) | 3.59 (0.95) |
| R-4 | 7.57 (1.23) | 6.24 (1.12) | 3.97 (0.87) | 3.93 (0.91) |
| R-5 | 7.25 (1.18) | 4.87 (1.02) | 3.17 (0.72) | 3.12 (0.84) |

* The figures in parentheses refer to the quantity excreted per gram of food intake.

declines considerably over a period of about 20 days, then more slowly for another 30 days, and thereafter increases slightly. This final increase can be visualized more clearly if one computes the value at 100 days. The excretion, for every 100 grams of body weight, would then have been 22.70 mgm. per day as compared with 20.94 mgm. on the 60th day. Although the figure 22.70 is actually a statistical abstraction, because the animals were not observed 100 days, it nevertheless shows what would in all probability have been found if the work had been continued that long. Support for this prediction can be found in previous work of the writer (7). In the work cited rats were deprived of large amounts of reserve protein by a sacrifice to fetal demands, and a gradual but un-

mistakable progressive increase in the urine N coefficient was demonstrated. The urine N coefficient is not a constant. There is only an approach to constancy. Perhaps a constant would be obtained if the weight of the skeleton were subtracted from the body weight.

The quantity of fecal N was determined on 4 pooled specimens for each rat. The 4 successive periods represented respectively 8, 12, 20 and a final 17 days on the diet. There was a progressive decline in the quantity of fecal N (table 1), and this cannot be ascribed entirely to the influence of a decrease in food intake, because the ratio fecal N/food intake decreased sufficiently to be of significance. A fraction of the fecal N is independent of food intake, and apparently as the animal continues eating the diet this fraction represents progressively a greater percentage of the total fecal N excretion. In the earlier work of Schneider (6) the existence of a fraction which is independent of food intake was also shown, but in contrast to the present confirmatory experiments, his conclusions were arrived at through a large number of short time feeding experiments.

## SUMMARY

When rats weighing 70 grams were taken from an adequate ration and fed a N-free diet a shift in urine N excretion followed the general equation $\log Y = a+b \log X$. The decline in body weight was in accordance with the equation $\log Y = a+b X$, and a slight progressive decline in the ratio fecal N/food intake was observed. The urine N coefficient declined over a period of about 50 days and thereafter increased slightly.

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# THE EXPERIMENTAL PRODUCTION OF OVULATION, LUTEINIZATION AND CYSTS OF THE CORPUS LUTEUM IN ADRENALECTOMIZED ANESTROUS CATS

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The cat and the rabbit usually ovulate only after mating. It has been demonstrated for the rabbit that coitus stimulates the anterior pituitary to secrete the gonadotropic hormones which induce ovulation (Fee and Parkes, 1929). The mechanism by means of which coitus accomplishes this is not entirely known. Brooks (1937) and Westman and Jacobsohn (1937) have reported that at one point in its course the coital stimulus passes through the infundibular stalk of the rabbit to the anterior pituitary gland. Others (Haterius and Derbyshire, 1937; Harris, 1937) have produced ovulation in the rabbit by stimulation of various localized areas in the hypothalamus. Rabbits ovulate after adrenalectomy if the operation is performed within one hour after mating (Friedgood, unpublished data, 1936; Brooks, 1938). It was found, however, that cats did not ovulate under similar experimental conditions (Friedgood, 1937). These observations have been repeated and confirmed; but, because of other experiments, a different interpretation than that originally suggested has been applied to these results. Although ether anesthesia alone, lasting ½ to ¾ hour, may not interfere with ovulation if it is started about 25 minutes after mating, cats do not ovulate if the abdomen is opened and a nonspecific operation (unilateral nephrectomy or partial splenectomy) is performed. It is therefore probable that the adrenals are not involved in the immediate impulse imparted to the pituitary during coitus. It is possible, of course, that the adrenal glands may directly or indirectly affect the process of ovulation in some other way, since clinical and laboratory studies suggest that dysfunction of these organs may seriously alter the physiological activity of the ovaries. The present investigation was undertaken to determine whether the ovaries are capable of responding to stimulation by the pituitary sex hormones after adrenalectomy of brief or long-standing duration. A preliminary report of these studies has already appeared (Friedgood and Foster, 1937).

[1] Aided by a grant from the Committee for Research in Problems of Sex of the National Research Council.

METHOD. Foster and Hisaw (1937) have demonstrated that the ovarian follicles of anestrous cats can be matured and ovulated by the proper administration of FSH and LH. The present observations were made on 13 normal anestrous cats which served as controls, and on 11 anestrous cats which were adrenalectomized during the experimental period. The 13 normal anestrous cats were injected subcutaneously twice daily for 4 days with FSH, and on the 5th day they received an intravenous injection of FSH and LH. The total daily dose of FSH was 0.4 gram equivalent and the intravenous dose consisted of 0.5 gram equivalent of FSH plus a 0.02 gram equivalent of LH. The FSH and LH were prepared according to the method of Fevold and Hisaw (1934) and the FSH was standardized so that 0.4 gram equivalent of FSH was equal to 1 R.U. These normal control animals were sacrificed at various intervals from 36 to 384 hours (16 days) after the last intravenous injection. Eight of the other 11 anestrous cats had a unilateral adrenalectomy 3 to 4 weeks prior to the beginning of injections with FSH. The second adrenalectomy was done during the last 2 days of subcutaneous injections with FSH. The remaining 3 animals were adrenalectomized 11 and 12 days before the intravenous injection of FSH and LH. They were supported by cortin and kept symptom-free throughout the experimental period. All of the adrenalectomized animals were autopsied at various intervals from 28 hours to 298 hours (12.4 days) after the intravenous injection of FSH and LH, which was given on the 5th day of the experimental period. Most of these animals showed different degrees of adrenal insufficiency before autopsy, but several of them, as noted above, were kept symptom-free by the injection of cortin in doses of 4 cc. twice daily. It had been determined previously that the injection of 15 cc. of this cortin per day in divided doses did not affect the ovarian follicles of estrous rabbits (unpublished data, Friedgood).

The ovaries from both series of cats were studied macroscopically and histologically; and measurements were made of the height of uterine and glandular epithelium and of the diameter of the uterine glands. The uteri were subjected to motility tests, i.e., observations of spontaneous motility and reactions to adrenalin. The motility technique was modified from that of Van Dyke and Hastings (1928). The bath fluid, prepared according to their method, consisted of a carbonate-phosphate buffered saline solution, the inorganic constituents and pH of which were identical with those of blood serum. The apparatus was suspended in a constant temperature oven at 38.6°C., with the pH and $CO_2$ tension maintained by bubbling the oxygen supply through sodium bicarbonate solutions. The bath fluid was freshly made for each test. The motility chamber contained 50 to 70 cc. of fluid. Observations were made on spontaneous motility, and on sensitivity to pituitrin (20 I.U. per cc.) and adrenalin (1:1,000

dilution) in dosages of 0.01 cc. and 0.1 cc., respectively. These dosages were decided upon in order that the hormones would be physiologically effective within the range of the volume of the motility chamber.

The adrenalin reversal reaction represents an alteration in the response of the cat's uterus to adrenalin, due to the influence of the corpus luteum hormone (van Dyke and Gustavson, 1929; Kennard, 1937). In describing this transition, inhibitory responses have been designated by − to −−−−, motor reactions by + to ++++, and mixed responses by combinations of + and − signs. The marked inhibitory response of the estrous uterus, for instance, is represented by −−−−. The change from an inhibitory to a motor response occurs at the onset of the lutein phase, and early luteinization is associated with a gradual loss of the marked inhibitory reaction of the uterus to adrenalin. During this phase slight motor responses may be followed by periods of inhibition (+− or +−−). The first definite motor response (+) has been taken as the time of the reversal to adrenalin. Progressive increase in the motor reaction of the uterus to adrenalin (+ to ++++) usually occurs in the course of several days.

Results. The administration of FSH and LH to normal anestrous cats according to the technique described, resulted in the growth of immature ovarian follicles, their ovulation and subsequent luteinization. Ovulation was noted in an unoperated animal sacrificed 36 hours after the intravenous injection of FSH and LH. These observations confirm those of Foster and Hisaw (1937). Ovulation and subsequent luteinization was found likewise in the adrenalectomized cats. Although ovulation of a few follicles occurred in the adrenalectomized cat autopsied at 30 hours, each ovum recovered from operated cats examined at 47 and 58 hours, respectively, still had an intact corona radiata and zona pellucida. This indicates that ovulation must have been delayed considerably, since the corona cells generally disintegrate within a few hours after ovulation.

This conclusion is fortified by results obtained from studies of the adrenalin reversal reaction in uterine motility tests. The adrenalin reversal reaction appeared in modified form (+−−) in 2 normal control animals at 47 hours and in 2 others at 48 and 50 hours after the intravenous injection of FSH and LH. A negative result was obtained in only 1 animal as late as 48 hours. Beginning with 60 hours, however, positive results were recorded consistently (table 1). The same test yielded negative results in adrenalectomized animals which were autopsied at 28, 30, 47, 58 and 70 hours. A positive response was found in 1 adrenalectomized cat at 64 hours, and was observed consistently only after a lapse of 75 hours or more. The animal whose uterus gave a + adrenalin reversal reaction was supported by cortin (table 2).

There was another significant difference between the reactions of the

uteri from two series of animals. Spontaneous motility of the uterus persisted until 96 hours after the intravenous injection of FSH and LH in the control series; whereas it disappeared with the occurrence of ovulation (at 47 hours) in the adrenalectomized cats. This probably signifies that there was a marked decrease in the secretion of estrin from the ovaries of the adrenalectomized cats. One would have expected the opposite result, namely, a prolonged estrin secretion because of the significant retardation of the process of luteinization.

TABLE 1

*Controls*

| ANIMAL NUMBER | HOURS AFTER INTRAVENOUS F.S.H. AND L.H. | SPONTANEOUS MOTILITY OF UTERUS | ADRENALIN REACTION | HISTOLOGIC RATING* |
|---|---|---|---|---|
| FH20 | 36 | ++++ | ---- | ++ |
| CNTrA | 42 | +++ | --- | ++ |
| CNB1A | 47 | Absent | +- | ++ |
| CN180A | 47 | +++ | +- | + |
| FH10 | 48 | +++ | --- | ++ to +++ |
| FH123 | 48 | +++ | +- | ++ |
| CNTrB | 50 | ++ | +- | ++ |
| FH17 | 60 | ++ | + | ++ to +++ |
| FH21 | 96 | + | ++ | ++ |
| CNTri | 120 | Absent | ++++ | No observations |
| FH18 | 144 | Absent | ++++ | ++++ |
| FH14 | 384 | Absent | ++++ | ++ |
| FH16 | 384 | Absent | ++++ | +++ |

TABLE 2

*Adrenalectomized animals*

| ANIMAL NUMBER | HOURS AFTER INTRAVENOUS F.S.H. AND L.H. | SPONTANEOUS MOTILITY OF UTERUS | ADRENALIN REACTION | HISTOLOGIC RATING |
|---|---|---|---|---|
| C52A | 28 | ++++ | --- | +? |
| C53A | 30 | +++ | --- | + |
| C136A | 47 | + | --- | + |
| C51 | 58 | 0 | - | + |
| CF2A* | 64 | 0 | + | No observation |
| C65B | 70 | 0 | -- | + |
| C50 | 75 | 0 | ++ | ++ |
| C136B | 96 | 0 | +++ | ++ |
| CF3* | 130 | 0 | +++ | ++++ |
| C115 | 143 | 0 | +++ | ++ to +++ |
| CF2B* | 298 | 0 | +++ | +++ |

Histological rating as given in table 1.

* Supported by 4 cc. cortin b.i.d. throughout the experiment. Adrenalectomized 11 and 12 days before intravenous injection of FSH and LH.

| RATING | HEIGHT OF UTERINE GLANDULAR EPITHELIUM | DIAMETER OF UTERINE GLANDS |
|---|---|---|
| | μ | μ |
| 0 (estrous state) | 10 to 14 | 20 to 30 |
| + | 18 to 20 | 45 to 60 |
| ++ | 20 to 30 | 60 to 80 |
| +++ | 30 to 40 | 80 to 120 |
| ++++ | Above 40 | Above 120 |

Little significance can be attached to the reaction of the uterus of the cat to pituitrin, since both series of animals yielded maximal responses to this hormone.

The adrenalectomized cats showed a modified response to the effects of FSH and LH in still another way, viz., the size and complexity of the uterine glands and the height to which the uterine and glandular epithelium grew. Measurements were made of the height of the uterine endometrial and glandular epithelium by means of an ocular micrometer which

was standardized against a stage micrometer by the usual method. The diameters of the neck and base of the neck and base of the uterine glands were also determined by this method. Measurements were made of 8 to 10 epithelial cells for each observation, and maximal and minimal diameters of the uterine glands were determined and recorded in a similar manner. According to these actual measurements, as abbreviated in the footnote to table 1, the normal pseudopregnant cats merited a ++ rating 36 hours after the ovulating dose of FSH and LH had been administered. With one exception, however, the adrenalectomized animals were not given a ++ rating until 75 hours had elapsed (table 2).

From 15 to 25 follicles were stimulated to growth and ovulation in each of the normal and adrenalectomized animals; but there were many other follicles which remained unruptured. Some of these became en-

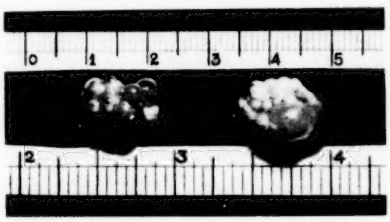


Fig. 1. The left ovary was removed from CF3 at 130 hours. Note the 3 fully cystic follicles on the right end of the ovary and 2 centrally liquefied corpora lutea near the middle of the gland. There is much solid lutein tissue to be seen at the extreme left and between the cystic follicles at the extreme right end. The right ovary was removed from CF2B at 298 hours. The solid lutein tissue is obvious along the top of the ovary, and 2 large cystic follicles (with luteinized walls) are clearly visible at the extreme right end of the gland. The upper ruler is graduated in centimeters, the lower, in inches. Compare these ovaries with cystic follicles in fig. 2. Foster and Hisaw, 1935.

larged to cystic proportions. Particularly striking in this respect were those removed from the adrenalectomized cats (fig. 1). The walls of these follicles were invariably completely luteinized, and their ova revealed maturation changes characteristic of the 1st polar body 2nd polar spindle stage. Another type of cystic follicle was also recognizable in both series of animals, viz., heavily luteinized follicles which appeared to be centrally liquefied. Most of these were lacking in ova and presumably had ovulated. The histology of both of these cystic structures is now being studied.

Conclusions. It is concluded from these experiments that the anterior pituitary hormones, FSH and LH, can induce ovulation in anestrous cats in the absence of the adrenal glands and their secretion. Uterine motility tests, measurements of uterine and glandular epithelium, and

measurements of the diameter of the uterine glands show that ovulation and functional luteinization are delayed in the adrenalectomized cats (tables 1 and 2). The development of extraordinary lutein cysts (fig. 1) also seems to be definitely associated with the absence of the adrenal glands. These huge lutein cysts were observed in the ovaries of cats showing adrenal insufficiency as well as in those adequately supported by cortin. It would appear, therefore, that a substance other than the life-sustaining hormone of the adrenal cortex is involved in their pathogenesis. We have not encountered a structure of similar proportions among the controls in this series, nor were such seen during previous experiments by Foster and Hisaw (1937), who used the same controls for another purpose. It is not feasible at present to attempt an explanation of these phenomena.

## SUMMARY

The ovarian follicles of 13 normal anestrous cats and 11 adrenalectomized anestrous cats were matured and ovulated by the administration of FSH and LH according to the method of Foster and Hisaw. The process of ovulation and of subsequent luteinization was delayed significantly in the animals which had been adrenalectomized prior to injection with FSH and LH. This was determined by histological examination of the recovered ova, by observation of the spontaneous motility of the uterus and of its reaction to adrenalin, and by measurements of the height of the uterine glandular epithelium and of the diameter of the uterine glands (tables 1 and 2). Corpus luteum cysts were found in the ovaries of both normal and adrenalectomized animals, but were much more striking in size in the latter group (fig. 1). These huge lutein cysts, measuring 5 to 7 mm. in diameter, were present in the ovaries of cats showing adrenal insufficiency as well as in those adequately supported by cortin.

The authors wish to thank Dr. David Klein of the Wilson Laboratories for supplying the adrenal cortical extract which was used in these experiments.

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# OBSERVATIONS ON THE BLOOD SUGAR LEVEL BEFORE, DURING AND AFTER HUNGER PERIODS IN HUMANS

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A considerable amount of work has been done in an attempt to determine any relationship between the blood sugar level and the cause of spontaneous gastric motility (hunger contractions). The evidence cited by Carlson in 1916 (2) appears to be the stimulus for the investigations which followed. In this early work, the author indicated that the blood sugar level was inversely related to hunger contractions.

Since that time, correlation of carbohydrate metabolism with the motility of the empty stomach has embraced several lines of study:

1. *Transfusion experiments.* Luckhardt and Carlson (3) showed that blood, transfused from starved animals and animals with pancreatic diabetes into normal animals, acted as a temporary stimulus to hunger contractions. At this time, the authors postulated the increase of *some* substance in the blood which acted as a stimulus to the motility of the empty stomach.

2. *Intravenous administration of glucose.* In 1924, Bulatao and Carlson (1) reported an inhibition of normal hunger contractions resulting from an elevation of the blood sugar level with intravenous glucose. Templeton and Quigley (12) reported a similar inhibition of the main stomachs of animals with Heidenhain pouches when dextrose was administered intravenously. On the contrary, Mulinos (5) was unable to find any inhibition of hunger contractions when glucose was given intravenously. Only in cases of hypermotility resulting from insulin was glucose effective in the inhibition of gastric contractions. In 1932 Quigley and Hallaran (7) reported an inhibition of hunger contractions of only 1 to 2 minutes' duration after the intravenous administration of glucose. Motility returned immediately, and blood sugar determinations showed a marked hyperglycemia. The same authors also followed the blood sugar level for a number of hours after intravenous glucose. They found that hunger contractions came on when the blood sugar level dropped below the normal, i.e., when glucose was being stored. However, they state that when compensation occurred and the blood sugar level rose, hunger contractions then decreased and stopped.

3. *Intravenous administration of insulin.* In the cases found in the literature where insulin was given, there is more unanimity of opinion. Bulatao and Carlson (1) showed some hypermotility of the empty stomach of the dog when insulin was injected. Regan (9) reported a primary depression followed by an increase in tonus and gastric activity when insulin was injected intravenously into fasting dogs. Wilder and Schlutz (13), Mulinos (5), Simici, Giurea and Dimitriu (11), Quigley, Johnson and Solomon (8) and others, all report that insulin causes an increase in the motility of the empty stomach. Hypermotility after the administrations of insulin seems to appear so consistently that many other workers have used this method of producing hypermotility to enable them to study the effects of other substances such as pituitrin (Quigley and Barnes, 6) and adrenalin (Wilder and Schlutz, 13) on the increased motility produced.

4. *Heidenhain pouches, vagus sections, etc.* Templeton and Quigley (12) in attempting to explain the mechanism by which insulin caused an increased activity of the empty stomach, resorted to the use of the Heidenhain pouch. They found that both subcutaneous and intravenous insulin inhibited the activity of the Heidenhain pouch and at the same time it augmented main stomach activity. Wilder and Schlutz (13) demonstrated that the hypermotility which followed subcutaneous insulin administration was rapidly abolished by atropine in doses of 0.5 mgm. given subcutaneously. During this period of atropine inhibition, the blood sugar level was even lower than that found during the insulin hypermotility. The same authors showed similar findings using adrenalin, i.e., the adrenalin inhibited the insulin hypermotility before there was any rise in the blood sugar level. Numerous authors, including Quigley and Hallaran (7), have also shown that insulin's action in increasing the motility of the empty stomach is destroyed when the vagus nerves are sectioned.

In undertaking this investigation, it was felt that whereas the aforementioned work was of considerable interest with reference to any interrelations between carbohydrate metabolism and hunger contractions, it did not answer the question, "Is the blood sugar level (whether low or high) responsible for the initiation of contractions of the empty stomach?" We feel that a better approach to a determination of the existence of any causal relationship of sugar level to hunger contractions is to determine the sugar levels of *normal humans* during a normal post-absorptive period and subsequently during hunger contractions, i.e., to see whether or not there is normally a fall (or rise) in the blood sugar level which precedes the onset of hunger contractions, and whether or not this level is altered after the hunger period is over and quiescence ensues.

Procedure. The conventional balloon-water manometer method of recording hunger contractions was used in this series. Six people who had had previous experience in swallowing balloons and recording hunger contractions served as subjects. In the majority of cases, these individuals

fasted for periods of 5 to 6 hours. The duration of fasting appeared sufficient, because in 90 per cent of the instances studied, a strong hunger period ending in incomplete tetanus was observed. Blood was drawn at the beginning of the hunger record and at 10 minute intervals thereafter until the completion of the "hunger period" and the onset of quiescence. Blood sugar determinations were made on all samples drawn, and hemoglobin determinations on the majority. The microchemical method of Miller and Van Slyke (4) was used in the determinations of the blood sugar, and the Sahli method for hemoglobin (10). In four of the experiments of the series reported, blood sugar determinations were made at 10 minute intervals for 2 hours preceding the first "hunger period" after a meal.

Results. The findings obtained in a series of 12 experiments on 6 human subjects are graphed in figure 1. One hundred and two blood sugar determinations (in duplicate) are shown on the graph. For purpose

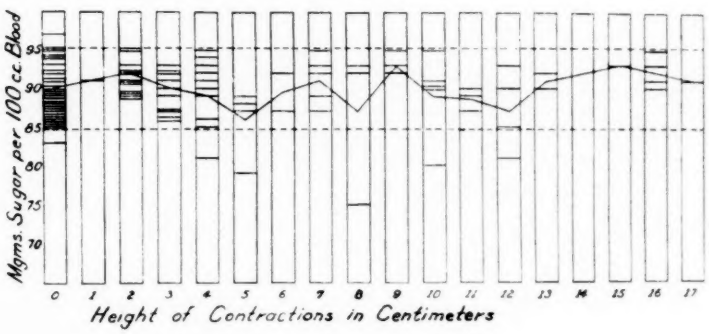


Fig. 1

of illustration, the blood sugar values in milligrams per cent are plotted against the height of the hunger contractions in centimeters. As indicated by the dotted horizontal lines, the blood sugar values with very few exceptions are seen to range between 85 and 95 mgm. per cent, which is within the range of experimental error of the method as determined by the authors. *This indicates that the blood sugar level is a straight line, and is unaltered by states of quiescence or motility of the stomach.* The blood sugar level during absolute quiescence, either before or after the "hunger period" was identical with that found during incomplete tetanus. Included in figure 1 is a line indicating the average blood sugar values for quiescence and all heights of contraction. The hemoglobin determinations made showed very little variation from sample to sample indicating little dilution of the blood samples.

When blood sugar determinations were made two hours before the actual running of the hunger record (four experiments) the same values

again were seen as were observed during the time the balloon was in the stomach. *At no time were there any consistently low (or high) values during the periods of quiescence.*

Figure 2 illustrates a representative "hunger graph," showing the length of fast for this subject, the actual hunger contractions, the time of sampling of blood and a curve drawn in to show the blood sugar values. For practical purposes, the blood sugar level is unaltered, although the subject shows a good "hunger period," preceded by quiescence and ending in incomplete tetanus. Previous recordings on this same subject have shown that this was his first "hunger period" following his breakfast. In addition, sugar values taken two hours before this graph are in the same range seen here, i.e., 90 mgm. per cent. Again, it is clearly seen that *there is no relationship between the blood sugar level and the state of activity of the empty stomach.*

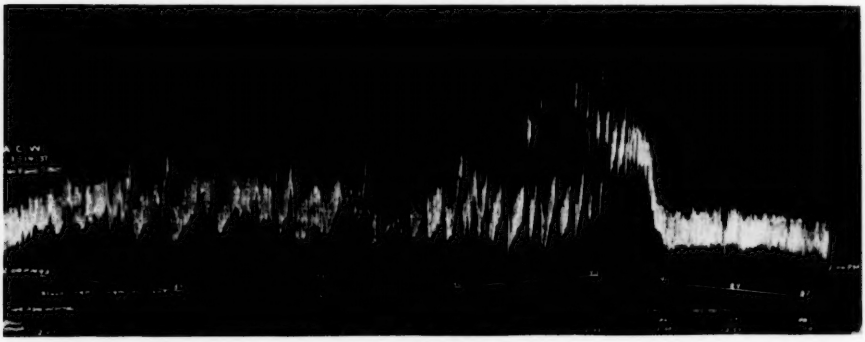

Fig. 2

Discussion. It has been shown that the blood sugar level of the fasting, human subject remains constant at the same time the empty stomach is undergoing changes in activity. If there was any causal relationship between the blood sugar level and hunger contractions this would not be the case. If a causal relationship existed, there should be a fall (or rise) in the level of the blood sugar preceding the onset of the contractions, and a subsequent rise (or fall) in the level after the contractions had ceased. This order of events would have to repeat itself before the succeeding hunger period. We could find no evidence for these hypothecations.

## SUMMARY AND CONCLUSIONS

1. Using the microchemical method of Miller and Van Slyke, blood sugar determinations were made *on human subjects*, preceding, during and after normal "hunger periods."

2. Within the range of experimental error (±5 mgm. per cent) no variations in the blood sugar level were found during the entire period.

3. A general conclusion to this work is that the level of the blood sugar bears no causal relation to the normal "hunger periods" of man.

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# THE EFFECT OF ALCOHOL ON THE HUNGER SENSE

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In 1914 Carlson and co-workers (2, 3) published their research on the action of stomachics on the gastric hunger mechanism. It was anticipated that these substances might augment hunger contractions since people had long used stomachics for improvement of poor appetite. On the contrary, it was found that hunger was not increased, but actually inhibited by alcohol or bitters. Conclusions seemed to indicate that alcohol stimulated the appetite while abolishing hunger. Cushny (4) in 1910 had proposed that stomachics acted mostly by suggestion on patients rather than by real drug action. Excluding the hunger mechanism, various investigators (5) have reported that alcohol causes an increased flow of gastric juice, while Moorhead (7) and Carlson (1) showed that various bitters had no influence on gastric secretion. In low concentrations alcohol is said also to decrease the emptying time of the stomach (Kast, 6).

The original purpose of this research was to continue the work of Carlson with alcohol in an attempt to determine more accurately the exact nature of alcohol in increasing the desire for food. It was thought that a greater insight might be obtained by *running experiments longer*, and analysing subjective sensations in more detail, since such a procedure might lead to a better understanding of the nature of appetite which at present is poorly understood. Perhaps, too, vigorous hunger contractions might follow the primary inhibition.

METHODS OF PROCEDURE WITH RESULTS. Hunger contractions were recorded by the usual balloon-in-stomach method, the balloon being connected with a water manometer writing on a kymograph record. The subject was placed in a comfortable chair with his back to the recording device so that he could not see the record. The subjects used were six healthy young males between the ages of twenty and thirty. The subject was to signal (recorded on the graph) whenever he had true hunger sensations, and was instructed not to signal for other sensations from the stomach nor for appetite. This meant that the subject had to practice signaling for hunger contractions for a time until he could always distinguish true hunger from other types of sensations. The method of signaling for hunger sensations consisted in having the subject press an

ordinary spring key in circuit with a signal magnet writing on the graph. In order to designate the various degrees of intensity of sensation, three spring keys in circuit with three separate signal magnets were used. One key, hereafter to be referred to as key I, was used to signal for sensations of mild or moderate hunger; a second, key II, represented strong hunger; the third, key III, being used to indicate hunger contractions that were experienced as disagreeable or even painful in intensity. The subject was instructed to press the key momentarily and then release it. He was then to press the key again as soon as he was sure that he still felt the hunger contraction, and so on. For example: At the beginning of a hunger contraction the subject would press the key and release it; then perhaps a second later, as the contraction became stronger he would press the key again. If the contraction continued to get stronger, it might be only a half second until he pressed the key for the third time. As the contraction would decrease in amplitude, the intervals between the signals would lengthen. In addition, if the hunger contraction were strong, the subject might start by signaling with key I as the contraction started, but as the amplitude of contraction increased he would change and signal with key II. If the contraction were very strong, he might then signal with key III. The reverse process would occur as the contraction gradually diminished and disappeared.

In the original procedure, it was decided to allow a hunger period to progress until approximately one-third finished, at which time alcohol diluted with water would be taken. The experiment would then be continued for a time considered sufficient to show what effect the alcohol would produce. The control experiments, according to the original plan, were to consist of an undisturbed hunger period with the subject recording his sensations. At the time, it was not considered advisable to interrupt the control records one-third through by drinking water equivalent in volume to the alcohol experiments, although this procedure was tried.

The strength of the alcohol solution and the total amount of alcohol to be taken by the subject was not decided on before undertaking the problem. Various amounts and dilutions were tried before a satisfactory value was obtained. Carlson, in his work, had used 50 to 100 cc. volumes in dilutions of 10 per cent with inhibition of the hunger period for one to two hours. The total quantity of alcohol amounted to 5 to 10 cc. This was considered to be too small a quantity of alcohol. It was thought that the amount should more nearly correspond to that of a cocktail or two, that is, enough alcohol to produce a moderate feeling of exhilaration or headiness. In other words, the quantity of alcohol should be equal to the amount taken ordinarily as an appetizer. In some of the experiments 100 cc. of 20 per cent alcohol was used but this seemed to be insufficient. Finally, 200 cc. of 20 per cent alcohol taken in two 100 cc. doses, about

five minutes apart, proved to be the desired amount. Consuming this quantity with the stomach empty, the effect begins to be noticed in about ten minutes; the peak of the action occurs about one-half hour after drinking, and the final effects of the alcohol last for three to four hours. It should be noted that this concentration and amount of alcohol was not distasteful to any of the subjects. Larger amounts of alcohol were taken but they proved excessive because the subject approached the state of drunkenness in which he could hardly keep awake or was unable to concentrate properly on his task.

TABLE 1

*Average values of first series of experiments*

| | CONTROL EXPERIMENTS | | | ALCOHOL EXPERIMENTS | | |
|---|---|---|---|---|---|---|
| | Subject 1 | Subject 2 | Average of all results | Subject 1 | Subject 2 | Average of all results |
| First twenty minutes of hunger period: | | | | | | |
| Average number of hunger contractions | 13.0 | 10.0 | 11.5 | 14.0 | 11.0 | 12.5 |
| Average height of hunger contractions (cm.) | 6.0 | 8.3 | 7.2 | 6.5 | 8.6 | 7.5 |
| Average sensation per hunger contraction | 10.1 | 11.0 | 10.6 | 15.0 | 14.1 | 14.6 |
| Period of quiescence in minutes | Continuous with first part | | | 38.0 | 45.0 | 41.5 |
| Remainder of hunger period: | | | | | | |
| Average number of hunger contractions | 31.0 | 21.0 | 26.0 | 34.0 | 26.0 | 30.0 |
| Average height of hunger contractions (cm.) | 8.7 | 12.3 | 10.5 | 7.5 | 10.6 | 9.0 |
| Average sensation per hunger contraction | 30.0 | 28.1 | 29.0 | 37.1 | 31.3 | 34.2 |
| Per cent of experiments showing tetany | 63.0 | 66.0 | 64.5 | 90.0 | 66.0 | 78.0 |
| Average duration of tetany in minutes | 7.5 | 4.0 | 5.7 | 5.6 | 5.0 | 5.3 |

Table 1 shows the results of the early experiments in which the hunger period was interrupted one-third the way through by the taking of alcohol. The intensity of the *hunger sensation* was calculated by dividing the total number of signals by the total number of hunger contractions. Signals from keys I, II, and III were given a value of one, two, and three respectively. These were added together to give the total number of signals. The results show an intensity of sensation 18 per cent greater in the alcohol experiments than the control. Looking at the records, however, one is impressed that the difference should have been much greater. In other words, the methods used evidently were masking the full effect of alcohol in increasing the *feeling of hunger*. It was decided that the explanation

of this lay in the fact that the control periods were uninterrupted so that the contractions following the first third of the period were undisturbed and consequently of good size. In the alcohol experiments, a period of inhibition always results after which the remaining two-thirds of the hunger period is finished. The onset of this last part of the hunger period after the alcohol interruption is marked by contractions which are small since the contractions do not commence at full amplitude but gradually build up again. So the method used to compare alcohol and control experiments partially obscured the alcohol effects, because the small hunger contractions at the onset of the last part of the period produce only mild sensations, whereas the corresponding contractions in the controls were large with consequently greater intensity of hunger.

It is also notable that the average height of the hunger contractions was greater in the controls, although a glance at the records showed no particular difference in height. This difference could be explained again on the basis that the *temporary inhibition* with alcohol and the gradual increase in amplitude thereafter caused a lowering of the average height of contractions since the control periods were not interrupted.

In view of the fact that the control experiments did reveal a higher average amplitude of hunger contractions, the greater intensity of hunger sensation in the alcohol experiments is all the more striking because *contractions averaging smaller were felt more strongly than large contractions.* It seemed apparent that if the average amplitude of contractions could be made the same in the control and alcohol tests, the intensity of sensation would be even greater in the alcohol experiments.

Since the comparison between the alcohol and control records was not perfectly satisfactory, it was decided that the procedure should be changed in order to obtain records of hunger contractions as nearly alike as possible experimentally. The results had shown that alcohol produced a much shorter period of inhibition of hunger than reported by Carlson, although in this work considerably larger doses of alcohol were taken. However, alcohol inhibition was not the purpose of this research so the decision was made to take either alcohol or water in equivalent volumes directly after starting the record and before the hunger period had begun. The volume would be 200 cc., using 20 per cent alcohol for the tests and tap water for the controls, in both cases at room temperature. After starting the record in this manner, it would be run until a hunger period developed and was completed. In addition, in order to simplify and increase the accuracy in determining the number of the hunger signals on the graphs, three automatic counters were connected in series with the three signal keys used by the subject to denote hunger sensations on the record. In the first series of experiments the hunger signals on the graphs were counted by using a small ten-power magnifying glass, which was necessary

because the signals often recorded very close together. The counting represented a laborious task and, undoubtedly, was not as accurate as an automatic counter. The type of counter used was the simple Veedor type actuated by means of a soft iron rod which was pulled through a coil whenever the signal key was pressed.

Table 2 represents the average of the results of all the experiments run in this second series. The new method of experimentation proved to be much more satisfactory in that the alcohol and control tests were practically alike, thus enabling a more accurate determination of the alcohol effect on the hunger sense. The most striking difference between the control and alcohol records is in the hunger sensations. The average intensity of hunger sensations in the alcohol graphs was 105 per cent greater than in the controls. In addition, when we compare the other

TABLE 2

*Average values of second series of experiments*

| | CONTROL EXPERIMENTS | | | | | | | ALCOHOL EXPERIMENTS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Subject 1 | Subject 2 | Subject 3 | Subject 4 | Subject 5 | Subject 6 | Average of all results | Subject 1 | Subject 2 | Subject 3 | Subject 4 | Subject 5 | Subject 6 | Average of all results |
| Average time elapsing between taking fluid and start of hunger period in minutes | 13.0 | 9.5 | 11.0 | 41.0 | 20.0 | 59.0 | 26.0 | 33.0 | 8.5 | 72.0 | 69.0 | 41.0 | 78.0 | 50.0 |
| Average number of hunger contractions | 37 | 28 | 37 | 46 | 30 | 37 | 36 | 49 | 54 | 39 | 19 | 22 | 43 | 38 |
| Average height of hunger contractions (cm.) | 7.3 | 7.8 | 6.5 | 5.4 | 9.2 | 7.7 | 7.3 | 6.5 | 9.4 | 7.8 | 10.4 | 4.8 | 6.6 | 7.6 |
| Average sensation per hunger contraction | 16.9 | 14.7 | 11.9 | 11.2 | 16.6 | 13.2 | 14.1 | 30.3 | 21.8 | 16.3 | 27.9 | 31.1 | 46.1 | 28.9 |
| Per cent of experiments showing tetany | 50 | 50 | 0 | 100 | 100 | 50 | 58 | 50 | 100 | 0 | 100 | 0 | 50 | 50 |
| Average duration of tetany in minutes | 5.5 | 5.0 | 0 | 9.0 | 6.5 | 12.0 | 7.4 | 5.0 | 5.5 | 0 | 4.0 | 0 | 9.0 | 5.9 |

factors in the table we notice only a slight difference. The hunger contractions appeared twenty-four minutes sooner after taking water than in the case of alcohol, whereas the period of alcohol inhibition in the first series averaged forty-one minutes. The number of contractions to the hunger period showed approximately the same average in alcohol and control experiments for the group as a whole, but there were individual variations in both directions. As had been expected, the average height of contractions in the alcohol series was much more similar to the control contractions than in series I.

DISCUSSION. These results show that alcohol has little effect on the hunger period except for some delay in the onset of the contractions. Once hunger has appeared, the contractions are so similar in alcohol and control tests as to be indistinguishable. However, the striking difference

lies in the degree of hunger experienced by the subject. *Alcohol markedly increases the intensity of the hunger sensations without increasing the height of the contractions.*

The fact that alcohol produced such an intensification of hunger in all subjects is strong evidence against suggestive effects, since the subjects were not informed as to any possible action of alcohol. In all cases, however, on being questioned after the experiments, the subjects were convinced that they felt the hunger much more intensely when alcohol was taken. This is further amplified by the fact that many small hunger contractions that passed unheeded in control experiments were easily recognized with alcohol. It is evident that some factor enhances the ability of the subject to feel hunger when alcohol is administered although the nature of this enhancement has not been determined. However, there appear to be three possible modes of action of alcohol which would intensify hunger sensations: 1, increased sensitivity of the nerve endings in the muscular layer of the stomach wall; 2, a process of facilitation or Bahnung of centers in the brain which experience hunger; 3, a depression of higher centers or higher functions of the brain with a relative increase in the importance of lower functions. It is thought that the third possible mode of action is the most applicable in this instance, because of the general agreement of investigators (8) that moderate amounts of alcohol act primarily by depression of the higher centers. If this is true, neither of the first two possibilities could operate. There is much in favor of this view, however. Many investigators agree that alcohol acts first on activities of the cerebral cortex which are developed or acquired through education, leaving more primitive sensations and emotions practically unaffected, at least temporarily. The hunger sense is presumably a lower type of sensation because of its universality and necessity. Hence, alcoholic depression of higher functions should produce a greater ability to sense the contractions of the empty stomach, that is, an intensification of the hunger sense.

So far the question of appetite has not been discussed. Carlson had concluded that the stimulating effect of alcohol must have been on appetite since hunger was inhibited. In this work, no method of measuring appetite was developed. If appetite is a true sensation, it is certainly more vague than hunger. In fact, appetite is not well understood, some disagreement existing as to its definition. However, the subjects were instructed to note what effect the alcohol had on the desire for food during the period preceding the onset of hunger contractions. Here again, in all cases, alcohol stimulated the desire for food. The first sensation produced by alcohol in the stomach was a peculiar feeling of warmth that was agreeable, but which was not interpreted as a desire for food. This sensation would disappear in less than five minutes and be followed by

a more vague feeling, definitely referred to the epigastrium, which was interpreted as a distinct desire for food. This epigastric sensation, difficult to describe, was continuous and less intense than hunger. The general opinion was that this feeling was one of moderate tenseness in the epigastrium, but an examination of the records revealed no increase in gastric tone. Some investigators might not consider this sensation to be appetite since they feel that appetite is not referred to the stomach at all but is experienced in the mouth and throat and is based on previous pleasant memories connected with food. Employing the term in this sense, these results could not be interpreted to mean that alcohol had increased the subject's appetite, since the sensation was definitely referred to the stomach region. However, since the sensation was one which was definitely interpreted as a desire for food, and since there were no hunger contractions present, there is apparently no choice except to pronounce this feeling appetite. Incidentally, in some of the subjects, this increased appetite sensation was interpreted as producing more desire for food than the hunger which followed.

It is seen from the above discussion that alcohol apparently has two actions: A preliminary one which increases the appetite, and a second action of intensifying hunger sensations. This can be interpreted in terms of the experiences of any one who has taken alcoholic beverages. It is a fairly common practice to drink a cocktail or two before eating dinner, partly because it seems to stimulate ability to eat and enjoy food. Now since the period between drinking and eating would ordinarily be too short for hunger to appear, the increased desire for food must be concerned with the first factor mentioned above, namely, appetite stimulation. This would be more reasonable than a stimulus to hunger sensation in view of the fact that as soon as food is eaten, hunger immediately ceases, whereas alcoholic beverages seem to stimulate one's desire for food for some time after food-taking has commenced. Consequently, the hunger intensifying action must operate under different conditions. This action could occur with the empty stomach, the individual taking a few drinks of liquor and not eating immediately afterwards. Thus a drink or two late in the afternoon or late at night would allow the hunger to return, to be felt more acutely than if no alcohol had been taken. It is a quite common experience that after attending a party during which time a few rounds of drinks are consumed one feels that he must eat before going to bed. This could be explained on the basis of the second action of alcohol and the action which has been determined in this experimentation although the appetite factor may not be entirely eliminated.

## SUMMARY

A method of recording the intensity of hunger sensations was developed which made it possible to study the action of alcohol in relation to hunger.

It was found that the hunger contractions, which occurred after the primary inhibitory effect due to the ingested alcohol, produced *hunger sensations* which were more intense, even slight hunger contractions producing hunger pangs compared with the inconsequential effect of similar contractions occurring in a control period. Alcohol did not increase the amplitude of hunger contractions. In addition, alcohol produced an epigastric sensation during the period of alcohol inhibition preceding the hunger contractions. This feeling was associated with an increased desire for food, but was less intense than hunger and continuous; consequently, it was considered to be appetite.

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# THE EFFECT OF CALCIUM ON THE OUTPUT OF SYMPATHIN IN THE FROG'S HEART

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Stimulation of the autonomic nerves is capable of setting free not only the organic substances acetylcholine and sympathin but also inorganic ions. Thus, the vagus, a cholinergic nerve, liberates potassium (Howell and Duke, 1908; Zondek, 1922; Asher, 1926). Also preganglionic fibers, which are cholinergic, set free potassium in sympathetic ganglia (Vogt, 1936). On the other hand, there is evidence that sympathetic stimulation causes calcium to appear (Lánczos, 1935; Gavrilescu and Jonescu, 1937). Furthermore, it has been pointed out by Zondek (1929) that KCl and $CaCl_2$ frequently mimic the effects of vagal and sympathetic stimulation, respectively. A few experiments have been done which suggest that potassium ions facilitate the liberation of acetylcholine from various tissues. Beznák (1934) demonstrated that acetylcholine was set free from frog hearts perfused with calcium-free Ringer's solution. Brown and Feldberg (1935, 1936) showed that KCl causes liberation of acetylcholine in a perfused superior cervical ganglion of the cat and that calcium inhibits this action. Feldberg and Guimarãis (1936) observed that KCl produces an increase in the acetylcholine content of the blood coming from the salivary glands, tongue and sweat glands of both cats and dogs.

In view of the antagonistic action of potassium and calcium on many structures and the frequently correlated antagonism of sympathetic and vagal stimulation, it seemed reasonable to suppose that if potassium mobilizes acetylcholine, calcium might bear a similar relation to sympathin. In order to test this possibility, the following experiments were undertaken.

METHODS. Hearts of winter frogs (October through December) were prepared for perfusion according to the method of Straub. The standard Ringer's solution used contained NaCl 0.7 per cent, $CaCl_2$ 0.02 per cent, KCl 0.02 per cent and $NaHCO_3$ 0.05 per cent. The stimulating current was supplied by a Harvard inductorium. The drugs used were adrenalin (Parke, Davis), atropine sulphate (Merck) and ergotoxine ethanesulfonate (Burroughs, Wellcome). In all cases, the hearts used were rendered

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hypodynamic by frequent washings with Ringer's solution before the experimental procedures were carried out.

RESULTS. A. *Liberation of a substance having a positive inotropic effect from a heart perfused with excess calcium.* The donor heart (D) was perfused with a Ringer's solution free from potassium but containing double the normal calcium content (0.04 per cent). The well-known positive effect of the calcium ion immediately appeared (fig. 1A). After one-and-a-half minutes the perfusate was removed and enough KCl added to give a concentration equal to the calcium present (0.04 per cent). The resulting solution was transferred to the recipient heart (R) which had previously been washed three times with a solution of similar inorganic composition.

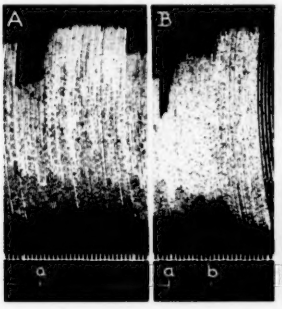


Fig. 1

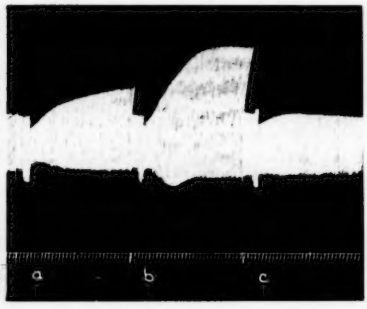


Fig. 2

Fig. 1. Donor heart (D). Hypodynamic, isolated from the frog. In this and the succeeding records the time signal marks 5-second intervals.

A. At *a*, Ringer's solution with $CaCl_2$ (0.04 per cent) but without KCl.

B. At *a*, same as in A; at *b*, start of stimulation of the vago-sympathetic trunk.

Fig. 2. Recipient heart (R). At *a*, perfusate from heart D (fig. 1A) balanced with 0.04 per cent KCl; *b*, same from heart D taken after *b* of figure 1B; at *c*, same as at *a*, but after heating for 2 minutes.

A marked increase in the amplitude of the contractions of heart R ensued (fig. 2, *a*).

In some experiments the vago-sympathetic trunk of heart D was stimulated during the perfusion, atropine (in a dilution of 1:100,000) being added to prevent the effect of the acetylcholine liberated (fig. 1B). Subsequent transfer of the solution as in the preceding type of experiment showed an even greater positive effect (fig. 2, *b*).

Various different concentrations of calcium and potassium were used to perfuse the donor hearts. All the solutions containing an excess of calcium over potassium liberated a substance having a positive effect on the recipient heart. Within limits, the greatest effects were recorded with solutions in which the greatest imbalance occurred, as in the first

experiment. Solutions containing equal amounts of the two substances were ineffective, even though both ions were present in considerable excess (0.04 per cent).

It was therefore concluded than an excess of calcium is capable of liberating from the frog heart a substance having a positive inotropic effect on a second or recipient heart. The following experiments were undertaken to determine certain physical and biological properties of the material.

B. *Physical and biological properties of the liberated substance.* One cubic centimeter of the high calcium (0.04 per cent), potassium-free Ringer's solution was perfused in the donor heart for 1½ minutes. The perfusate was then removed to a test tube and heated to a point just below boiling

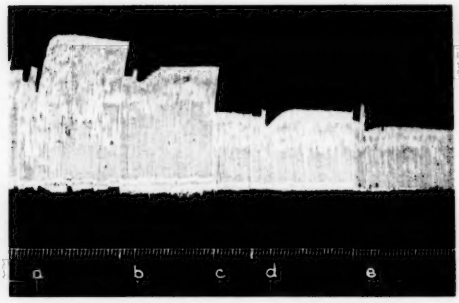


Fig. 3

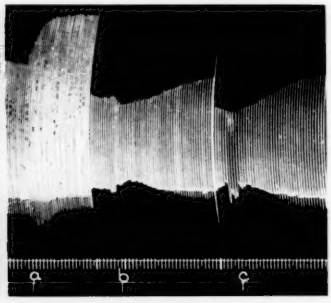


Fig. 4

Fig. 3. Heart R. At *a*, adrenalin (1:100,000,000); at *b*, perfusate as in figure 2, *a*; at *c*, after ergotoxine for 2 hours; at *d*, adrenalin (1:1,000,000); at *e*, perfusate as in figure 2, *a*.

Fig. 4. Heart R. At *a*, perfusate as in figure 2, *a*; at *b*, same solution after 2 hours of dialysis of 3 cc. against 3 cc. of Ringer's solution of the same inorganic concentration; at *c*, dialysate from *b*.

for 2 minutes. It was then cooled to room temperature by running water and KCl was added to a concentration of 0.04 per cent. The resulting solution caused no change in the recipient heart (fig. 2, *c*). As might be expected from the foregoing experiment, ashing the perfusate, and subsequently making the ash up to the original volume with distilled water and adding KCl to give a concentration of 0.04 per cent, also resulted in a solution without a positive effect on heart R.

If, instead of heating the solution, oxygen was bubbled through it for 5 hours, the stimulating substance was destroyed. It was therefore concluded that the substance is of organic nature, oxidizable and thermolabile.

C. *Effect of ergotoxine.* If the recipient heart was washed with Ringer's solution containing ergotoxine (1:100,000) until the effect of even high

concentrations of adrenalin (1:1,000,000) was nearly abolished (fig. 3, *d*), subsequent perfusion with the test solution obtained from the donor heart D, as in the first type of experiment, was ineffective (fig. 3, *e*).

D. *Dialyzability of the substance.* The perfusates from several successive perfusions of the donor heart with the high calcium, potassium-free solution were collected and enough KCl added to make a concentration of 0.04 per cent. The resulting solution was then dialyzed by the Loewi (1936) method against Ringer's solution of the same inorganic composition. After 2 hours the dialysate acquired the ability to produce a positive effect on the recipient heart (R). Such an experiment is illustrated in figure 4.

## CONCLUSIONS

From the results reported above it appears that perfusion of a donor heart with Ringer's solution containing a calcium excess, unbalanced by potassium, results in the liberation of a substance having a positive inotropic effect on a second heart. The action of this substance is additive with that of the accelerator substance liberated by stimulation of the vago-sympathetic. The material is oxidizable, is destroyed by ashing or simple heating to near the boiling point for a few minutes, and it passes easily through a dialysis membrane. Its effect on a recipient heart is abolished by ergotoxine.

These properties are similar to those described by Loewi (1921, 1924, 1936) for the accelerator substance liberated by sympathetic stimulation. It is therefore concluded that calcium sets free sympathin from the frog's heart.

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# THE EXCRETION OF ENDOGENOUS "CREATININE" BY THE HUMAN KIDNEY

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Diversity of opinion still exists concerning the nature of the material in blood responsible for the Jaffé reaction although Miller and Dubos (1937a) have introduced a new analytical technic which indicates that nearly all of it is actual creatinine. The question has some bearing on the mechanism of renal activity because, so long as it is not known how much of this chromogen is true creatinine, there exist three possible methods of calculating actual plasma creatinine clearance. One may, as is usually done, avoid the problem by artificially raising the plasma creatinine concentration to such levels that error from this source disappears; the formula is:

$$(1) \qquad \frac{\text{Total urine chromogen, mgm./min.}}{\text{Total plasma chromogen, mgm./cc.}}$$

At near normal blood values, however, such a scheme may entail considerable error, avoidable by use of the formula:

$$(2) \qquad \frac{\text{Exogenous urine chromogen, mgm./min.}}{\text{Exogenous plasma chromogen, mgm./cc.}}$$

This permits theoretically accurate estimations at low plasma concentrations but of course requires preliminary determination of the endogenous "creatinine" clearance. Finally the clearance of true creatinine can be achieved with or without creatinine ingestion by the formula:

$$(3) \qquad \frac{\text{Total urine chromogen, mgm./min.}}{\text{Total plasma chromogen—non-creatinine chromogen, mgm./cc.}}$$

It is the purpose of this paper to present evidence which indicates that all but about 0.5 mgm. per cent of the total chromogenic substance in human plasma is excreted by the kidney as though it were exogenous creatinine.

Information regarding the relationship between the concentration of creatinine in human plasma and its rate of urinary excretion is surprisingly

meager and inconsistent. MacKay (1930) and Medes and Berglund (1935) found that when these values were plotted against each other they fell along a straight line which went through the origin, and Shannon (1935), from data obtained by raising plasma creatinine concentration to very high levels, inferred that this was true. In another subject reported by Medes and Berglund, however, and in the one recorded by Cope (1931) this line was such as to indicate that when urinary excretion of creatinine reached its hypothetical zero there must still have been a small amount (less than 1 mgm. per cent) of chromogenic material remaining in the plasma. Jolliffe and Chasis (1933) found no relation between creatinine clearance and plasma concentration between the levels of 1.8 and 11.6 mgm. per cent but present no figures concerning the excretion of endogenous chromogen. In those studies which do include such information it is apparent that the clearance of "endogenous" creatinine is significantly lower than that obtained with artificial plasma concentrations (Shannon, 1935; Medes and Berglund, 1935; Hayman, Halsted and Seyler, 1933). Because of these discrepancies we have investigated this relationship in 4 normal subjects and 2 nephritics with results which, in confirmation of Cope, suggest that there is in human plasma about 0.5 mgm. per cent of chromogenic substance which does not appear in bladder urine.

METHODS. The endogenous "creatinine" clearance was determined in all subjects by averaging several observations obtained on successive days; the results were remarkably constant in a given individual regardless of the rate of urine flow. The clearance of total chromogenic material was then obtained in the usual way after the ingestion of variable amounts of creatinine. Frequently full water diuresis was established but again no correlation was observed between plasma clearance and the urine minute volume. Urine was usually collected at 20 minute intervals but occasionally the periods were extended to as long as 4 hours in order to provide a greater fall in successive plasma values. Creatinine determinations were made by the method of Folin and Wu; most of the plasma values were obtained from tungstic acid filtrates of oxalated blood (Peters and Van Slyke, 1932) but a few were done on the iron filtrate of Steiner, Urban and West (1932). Simultaneous analyses on the same blood sample gave equivalent results with both filtrates. Table 1 illustrates the recovery of added creatinine from iron filtrates of normal serum, the theoretical value having been unknown to the analyst.[1]

On 15 watery solutions of creatinine the mean analytical error was $-1.9 \pm 3.9$ per cent.

RESULTS. Figure 1 illustrates the observed relationship between the concentration of total chromogenic substance in the plasma and its rate of renal excretion. There is some scattering of course but in no case can

[1] I am greatly indebted to Carol B. Kaminer for some of these determinations.

a line be drawn which will extend through the endogenous value to the intersection of the axes, a phenomenon suggesting that about 0.5 mgm. per cent of plasma chromogen is either not available for glomerular filtration or that it is reabsorbed by the renal tubule.

It then became of interest to apply this value to the actual clearance figures. Since the clearance of exogenous creatinine seems to be open to fewer theoretical objections than either of the other formulae we have used it as a basis for comparison. Cope (1931) had noticed that, whereas the clearance of total chromogenic substance declined as the plasma concentration approached normal levels, it became virtually constant if 0.5 mgm. per cent were arbitrarily subtracted from the observed plasma

TABLE 1

| INITIAL | CHROMOGENIC MATERIAL, MGM. PER CENT | |
|---|---|---|
| | Expected | Found |
| 1.1 | 12.8 | 12.4 |
| 1.1 | 8.6 | 8.7 |
| 1.1 | 4.3 | 4.5 |
| 1.6 | 9.8 | 9.4 |
| 1.6 | 7.7 | 7.6 |
| 1.6 | 5.8 | 5.9 |
| 1.6 | 4.6 | 4.5 |
| 1.6 | 4.1 | 3.9 |
| 1.6 | 3.6 | 3.3 |
| 1.6 | 2.9 | 3.0 |
| 1.6 | 2.3 | 2.2 |
| 1.6 | 1.9 | 2.0 |

values. In figure 2 we have arranged our data so that the open circles represent the ratio

$$\frac{\textit{Exogenous clearance}}{\textit{Corrected clearance}}$$

where the denominator is calculated by utilizing total chromogenic substance in urine but subtracting 0.5 mgm. per cent from this value for plasma. It is obvious that over a range of 1.6 to 13.8 mgm. per cent total plasma chromogenic substance the two clearances are virtually identical and quite independent of plasma concentration. In contrast to this uniform behavior is the manner in which the clearance of total chromogenic material falls as the endogenous level is approached; within the range studied the ratio

$$\frac{\textit{Exogenous clearance}}{\textit{Total chromogenic clearance}}$$

(solid dots) is with one exception always greater than unity and steadily increases as plasma concentration falls. Below 6 mgm. per cent this is a significant deviation.

Illustrative of the precise manner in which the corrected and exogenous clearances duplicate each other are the figures in table 2 which form the basis for the curve of subject *F* in figure 1.

Discussion. Chemical evidence concerning the existence of true creatinine in blood need not be discussed here except to point out that it

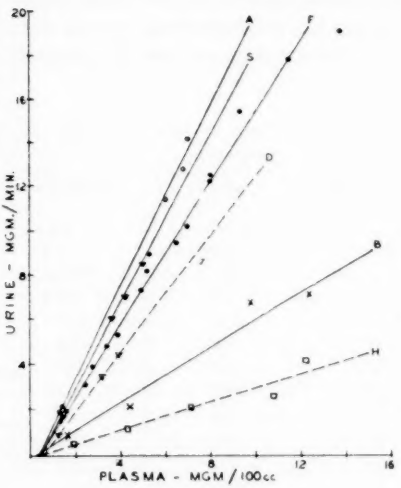


Fig. 1

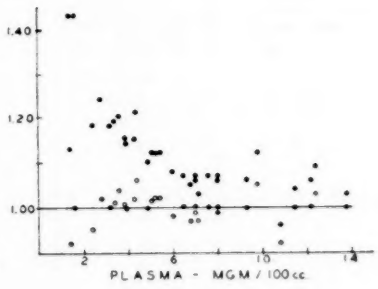


Fig. 2

Fig. 1. The excretion of total chromogenic material. The interrupted lines *D* and *H* were obtained from patients with glomerulonephritis. Subject B was clinically normal, and his diminished clearance is unexplained.

Fig. 2. The relation between plasma creatinine and plasma clearance. Solid dots represent the ratio $\frac{\text{Exogenous clearance}}{\text{Total chromogenic clearance}}$, open circles the ratio $\frac{\text{Exogenous clearance}}{\text{Corrected clearance}}$.

is as yet inconclusive. Physiological evidence is equally indecisive and it is not claimed that the above data constitute direct proof. The fact that the clearance of total chromogenic substance becomes independent of plasma concentration and identical with the exogenous clearance only when the former is corrected for that fraction of plasma chromogen which apparently does not appear in bladder urine may be mere coincidence. It lends circumstantial support, however, to the finding of Goudsmit (1935) that enough creatinine is extracted from plasma during passage

through the kidney to account for all that is found in urine. Hayman, Johnston and Bender (1933) found that the chromogenic material released from Lloyd's reagent was slightly less than the total amount originally present in serum, and when the

$$\frac{\textit{Exogenous clearance}}{\textit{Total chromogenic clearance}}$$

ratios are recalculated from the data of Cope (1931) and Medes and Berglund (1935) as in figure 2, the curves are very similar to ours. Since there is no reason to believe that creatinine is reabsorbed by the renal tubule it appears that these unrelated observations can best be explained

TABLE 2

| CREATININE | | CLEARANCE | | |
|---|---|---|---|---|
| Plasma | Urine | Total chromogenic | Corrected | Exogenous |
| *mgm. per cent* | *mgm. per minute* | | | |
| 1.37 | 1.44 | 105 | 166 | |
| 1.6 | 1.96 | 122 | 178 | 177 |
| 2.46 | 3.04 | 124 | 155 | 147 |
| 2.8 | 3.9 | 139 | 169 | 172 |
| 3.4 | 4.8 | 141 | 166 | 168 |
| 3.9 | 5.3 | 136 | 156 | 157 |
| 4.95 | 7.25 | 147 | 163 | 162 |
| 5.2 | 8.2 | 159 | 175 | 178 |
| 5.3 | 9.0 | 170 | 187 | 191 |
| 6.5 | 9.5 | 146 | 158 | 157 |
| 7.0 | 10.2 | 146 | 157 | 157 |
| 8.0 | 12.2 | 153 | 163 | 162 |
| 8.0 | 12.5 | 156 | 167 | 166 |
| 9.3 | 15.5 | 166 | 176 | 176 |
| 11.5 | 17.9 | 156 | 163 | 162 |
| 13.8 | 19.2 | 139 | 144 | 143 |

by assuming that most, but not all, of the Jaffé-reactive substance in human plasma is actually creatinine. Furthermore, the uniform excretory rate of endogenous creatinine and the constancy of these

$$\frac{\textit{Exogenous clearance}}{\textit{Corrected clearance}}$$

ratios strongly suggest that tubular secretion cannot play a significant rôle in the process of creatinine elimination. Claims to the contrary are based largely upon the data of Shannon (1935) but we know of no proof that inulin is not reabsorbed by the human tubule and regard the creatinine concentrations used by him as far beyond the physiological range.

The data in figure 1 from the 2 nephritics do not support the claim of Miller and Dubos (1937b) that there is a great increase in the non-creatinine fraction of plasma chromogen in nephritis; only one of them however, had appreciable renal insufficiency.

## CONCLUSION

Plasma creatinine clearance is not absolutely independent of plasma concentration but is influenced by about 0.5 mgm. per cent of chromogenic substance which does not pass from plasma to bladder urine. Reasons are given for the belief that the tubules of the human kidney probably do not secrete creatinine.

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# THE EFFECT OF COMBINED THYROIDECTOMY AND GONADECTOMY ON COMPENSATORY ADRENAL HYPERTROPHY IN UNILATERALLY ADRENALECTOMIZED RATS

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The present experiments were carried out with the idea of gaining some evidence as to what histological type of cell in the pituitary is responsible for growth of the adrenal cortex. The data were presented in brief before the American Physiological Society (13). It was thought that if the pituitary were radically altered in its cellular make-up, qualitatively and quantitatively, by means of thyroidectomy combined with gonadectomy (14), and one adrenal were removed and compensatory hypertrophy of the remaining adrenal studied, then a study of the histological appearance of the pituitary in such animals might give some indication of the type of cell in the pituitary that is responsible for adrenal growth.

EXPERIMENTAL PROCEDURE. Use was made of male and female rats that were born in this laboratory of an inbred colony that has been maintained for 4 years. They were fed on table scraps supplemented with Purina. Thyroidectomy, gonadectomy, and combined thyroidectomy and gonadectomy were carried out at 5 weeks of age. In some rats the left adrenal was removed at the same time, in others the left adrenal was removed 4 weeks later, and in others both adrenals were left intact. Control rats had thyroids and gonads left intact but had the left adrenal removed at 5 or 9 weeks of age, and others had all organs intact. All rats were killed at 16 weeks of age. At death the adrenals, kidneys and pituitaries were weighed. The ratio of adrenal weight to kidney weights was calculated. Kidney weights were thought to be a fairer index of visceral growth than body weights, as the subcutaneous tissues are disproportionately increased after thyroidectomy or gonadectomy and this consequently introduces a large error in the values for body weight. The pituitaries were fixed in Helly's fluid and stained with Mallory's acid fuchsin-orange G-aniline blue method. Complete thyroidectomy was evidenced by marked increase in weight of pituitary (14), characteristic histological changes in the pituitary (11, 14) and retardation of growth of kidneys.

RESULTS. Figures 1 and 2 give all the pertinent data of values for the ratios of adrenal to kidney weights, the number of animals used, the amount of variation in each group, and mean values. In the normal female there is a wide range of adrenal weights due to oestrual cycles, a variation which is well known (1). Since the thyroidectomized rats are stunted in growth by that operation, the ratio of adrenal to kidney weights was used

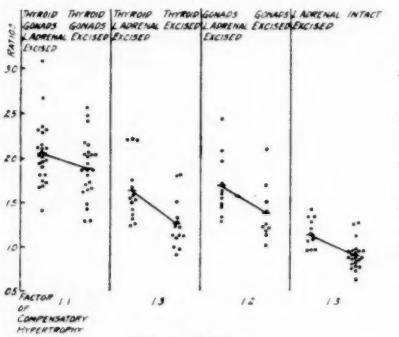


Fig. 1. Males

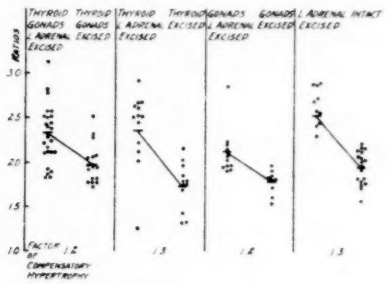


Fig. 2. Females

Fig. 1. Ratios of adrenal weights to kidney weights. Males. In each of the 4 groups divided by vertical lines, the right hand column represents rats with adrenals intact and the values charted represent

$$\frac{\frac{1}{2}\text{ weight of both adrenals}}{\text{weight of kidneys}} \times 100;$$

and the left hand column in each of the 4 groups represents unilaterally adrenalectomized rats and the values charted represent

$$\frac{\text{weight of remaining right adrenal}}{\text{weight of kidneys}} \times 100.$$

The means in each group are represented by short horizontal lines. Lines are drawn between the means in each pair to enable the eye to follow more readily the comparison. The factor of compensatory adrenal hypertrophy is

$$\frac{\text{the mean in unilaterally adrenalectomized rats}}{\text{the mean in rats with adrenals intact}}$$

the value for which is represented at the foot of each group.

Fig. 2. Ratios of adrenal weights to kidney weights. Females. This chart is constructed on the same plan as figure 1.

in this chart instead of the absolute weights of adrenals. It will be seen that in the rats with both adrenals intact (right hand columns) this ratio in males is increased after thyroidectomy or gonadectomy and is greatest after the combined operation; while in females this ratio is not greatly altered. It will be seen that compensatory adrenal hypertrophy occurs after combined thyroidectomy and gonadectomy, but may be found to a lesser extent than in other groups.

Table 1 gives the absolute weights of adrenals and kidneys in the different groups. When both adrenals are intact, in males there is increased weight of the adrenals after castration but thyroidectomy has no significant effect; in females thyroidectomy is associated with lower weights of the adrenals. Compensatory hypertrophy calculated from absolute weights shows less hypertrophy after combined operations in the males.

TABLE 1

| | MALES | | | | FEMALES | | | |
|---|---|---|---|---|---|---|---|---|
| | Thyroidectomy and gonadectomy | Thyroidectomy | Gonadectomy | Control | Thyroidectomy and gonadectomy | Thyroidectomy | Gonadectomy | Control |
| In rats with left adrenalectomy: | | | | | | | | |
| Mean weight of right adrenal (mgm.) A. | 25.4 | 19.7 | 30.2 | 23.3 | 27.4 | 26.4 | 38.0 | 40.7 |
| Standard deviation | 6.4 | 4.1 | 6.0 | 2.8 | 6.3 | 7.9 | 5.5 | 3.9 |
| Number of rats | 24 | 14 | 11 | 11 | 26 | 13 | 11 | 10 |
| Mean weights of both kidneys (grams) | 1.2670 | 1.2444 | 1.7158 | 1.9944 | 1.1904 | 1.1021 | 1.7896 | 1.6165 |
| In rats with both adrenals intact: | | | | | | | | |
| ½ weight of both adrenals—mean—(mgm.) B. | 23.2 | 16.6 | 24.9 | 17.7 | 23.6 | 18.4 | 31.7 | 31.3 |
| Standard deviation | 4.3 | 2.9 | 4.4 | 2.4 | 4.9 | 4.2 | 4.9 | 4.3 |
| Number of rats | 24 | 14 | 10 | 21 | 17 | 12 | 9 | 19 |
| Mean weights of both kidneys (grams) | 1.2648 | 1.3844 | 1.8381 | 1.9848 | 1.1994 | 1.0622 | 1.7305 | 1.5819 |
| Compensatory adrenal hypertrophy: $\frac{A}{B}$ | 1.05 | 1.18 | 1.21 | 1.31 | 1.16 | 1.43 | 1.19 | 1.30 |

As compensatory adrenal hypertrophy occurs rapidly, there were no significant differences in the results in rats that had had adrenalectomy done at 7 and 11 weeks before death.

Although the diet was not standardized, the body weights of the control rats were comparable to the weights given in Donaldson's tables for normal rats of the same age; kidney weights of those with adrenals intact were closely similar to those with unilateral adrenalectomy, in each group; and when the ratio of adrenal to kidneys is taken rather than absolute

weights, variations in kidney growth within any one experimental group becomes unimportant.

Discussion. Data on the effect of gonadectomy on the weight of adrenals have been given by Andersen and Kennedy (2), Freudenberg and Howard (7), Freudenberg and Hashimoto (6), Lawless (8), Billeter (3), and others. Data on the effect of thyroidectomy on the weight of adrenals have been previously discussed (15). Winter and Emery (10) have recently described compensatory adrenal hypertrophy of normal, castrated, and thyroidectomized rats. Their experiments were done from the point of view of showing that compensatory adrenal hypertrophy can occur in the absence of the thyroid and is a direct effect of the pituitary upon the adrenal. Their method of calculation is different from mine. They do not give data on thyroidectomized females. No data have been found in the literature on the effect of combined thyroidectomy and gonadectomy on compensatory adrenal hypertrophy.

Considering the fact that there are many secretory principles elaborated by the pituitary and yet only 2 secretory types of cells are recognized by histologists, either a cell of a given histological type is secreting principles that stimulate more than one peripheral endocrine organ, or we are not recognizing all the histological types of secretory pituitary cells that really exist.

When a peripheral endocrine organ is diseased or ablated, the pituitary reacts by concrete cellular changes. After castration, "castration cells" develop; after thyroidectomy, "thyroidectomy cells" appear which show distinct histological differences from "castration cells." Presumably the cell that reacts in consequence of the loss of a peripheral endocrine organ is the pituitary cell that is producing the hormone that affects the endocrine organ in question. When there is no peripheral organ to act upon, probably the pituitary secretion controlling that end organ is accumulating unused in the pituitary cell. For instance, "castration cells' have been correlated with increased gonadotropic hormone content of the castrate pituitary. Similarly after thyroidectomy, the pituitary contains an abundance of thyrotropic hormone (12). In experiments in progress, while castration cells are prevented from developing by oestrone injections, as is well known, thyroidectomy cells are found to be not at all inhibited by the same doses of oestrone. Thus thyroidectomy cells and castration cells appear to be different both histologically and functionally.

The pituitary cell that produces adrenocorticotropic hormone is not known. Since complete bilateral adrenalectomy is so rapidly fatal, there probably is not time for a pituitary cell to react, and no "adrenalectomy cells" have been described, although various investigators have found a variety of histological changes within basophiles in patients with Addison's

disease (5). Since after removal of one adrenal, compensatory hypertrophy of the remaining adrenal occurs only when the pituitary is present (4, 9), it is reasonable to search in the pituitary for a cell that is responsible for adrenal growth.

In the experiments on compensatory adrenal hypertrophy here reported the pituitary was radically altered in cellular pattern. After the combined thyroidectomy and gonadectomy, as I have described previously, approximately 90 per cent of all cells are either "thyroidectomy cells" or "castration cells," which can easily be distinguished from each other (14). Since compensatory adrenal hypertrophy bears no relation to the numbers of these cells, both of these types of cells can be excluded as the elaborators of the adrenocorticotropic hormone. Thyroidectomy causes a loss of nearly all acidophiles, which are the most numerous cells in the normal pituitary. Since compensatory adrenal hypertrophy occurs in spite of this loss, the acidophile can be excluded as the elaborator of the adrenocorticotropic hormone. The only remaining cells are a few chromophobes, which are generally accepted as nonsecretory precursors of secretory cells, and non-vacuolated basophiles which have not reacted to either thyroidectomy or gonadectomy. By a process of exclusion, the latter basophiles are indicated as those that control adrenal growth. In the individual rats in which compensatory hypertrophy seemed to be slightly less after gonadectomy combined with thyroidectomy, this would probably be accounted for by the crowding of the pituitary by abnormal cells which probably reduces the activity of the cells responsible for adrenal growth.

Although in any normal human or animal pituitary, great variations in the histological characters of the basophiles may be seen, these variations have generally been ascribed to different stages of secretory activity, the more deeply staining granular cells being regarded as ripe cells. Perhaps some of these variations in morphology really represent types of basophiles that are forming different hormones. From this point of view, it would be conceivable that in the normal pituitary different basophilic cells can not be distinguished from each other, but that when a peripheral endocrine organ is ablated, basophilic cells can then be dissociated. Thus both a functional and histological difference becomes apparent. It seems impossible at present to secure evidence as to the ultimate derivation of these different types of basophiles; that is, whether different types of basophiles develop independently from a precursor chromophobe or whether an existing basophile may alter its form under different conditions.

However that may be, the experiments show that adrenal growth may be dissociated from the growth of other endocrine organs. Whatever cell produces the adrenocorticotropic hormone, it seems to function independently of the gonads and thyroid. When this function seems to be

reduced, this impairment can be accounted for by the crowding of the pituitary by pathological types of cells.


## SUMMARY

1. The ratio of adrenal to kidney weights in male rats is increased after thyroidectomy, gonadectomy, or after combined thyroidectomy and gonadectomy; it is not significantly altered in females.

2. When one adrenal is removed, compensatory hypertrophy of the remaining occurs after either thyroidectomy, or gonadectomy, or combined thyroidectomy and gonadectomy.

3. The acidophiles, "castration cells" and "thyroidectomy cells" of the pituitary can be excluded as cells controlling adrenal growth, since their numbers bear no relation to adrenal weights.

4. By exclusion, it seems likely that adrenal cortical growth is controlled by a basophilic cell that is not a "castration cell" or a "thyroidectomy cell."

5. Adrenal growth is not accomplished through the mediation of the thyroid or gonads.

6. When compensatory adrenal hypertrophy is found to a lesser extent it may be accounted for by the crowding of the pituitary by pathological types of cells.


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# THE UTILIZATION OF β HYDROXYBUTYRIC ACID BY THE ISOLATED MAMMALIAN HEART AND LUNGS

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The problem of ketone body utilization by muscular tissues is important because of its bearing on the problem of the direct utilization of fat by muscle. The question has already been studied by Snapper and Grünbaum (1928) in striated muscle, in which it was found that 45 to 75 per cent of added ketone was destroyed without acetone formation. We are aware of only one previous attempt to study this question in connection with heart muscle, which was by Goldfarb and Himwich (1933) who proposed to solve the problem by a study of arterio-venous ketone differences. Their results were inconclusive, since blood ketone determinations are subject to a considerable random error. These authors stated that they considered changes of ±0.8 mgm. per cent significant. In a table of arterial blood values they indicate that they worked with concentrations as low as 5 mgm. per cent. Their random error was 32 per cent of this value. Obviously it could not be expected that heart muscle would remove this fraction of the total blood ketone in one circuit through the organ. We believe therefore that without further evidence their conclusion that the heart muscle adds ketone bodies to the blood under certain unspecified circumstances is unwarranted.

In order to avoid the difficulties inherent in metabolism studies by the arterio-venous difference method we have performed experiments with isolated perfused organs in which blood can be recirculated for long periods of time and the concentration changes made so large as to render the admittedly large analytical error in blood ketone analysis of no importance to the general conclusions.

METHODS. Heart lung preparations of the usual type were prepared as by Peters and Visscher (1936) using dogs and goats. In addition experiments were performed in the same general way with isolated dog lungs perfused with blood by an all glass and rubber pump system delivering 500 cc. of blood per minute to the pulmonary artery which was cannulated. The venous blood escaped through the incised veins into a glass reservoir from which it was withdrawn and recirculated by the pump. The lungs were aerated and the oxygen consumption measured

as described in the above citation. Blood ketones were determined by the Barnes (1937) method. Absolute balances were obtained by determining the ketones in the tissues at the termination of each experiment by grinding the tissues and taking random samples for analyses. The several samples agreed with one another in general, but these figures are less accurate than those for blood. The resulting error is minimized by the fact that in general the blood volume was between four and five times the tissue mass. The tissue concentration of ketone bodies was approximately the same as the blood level and although our calculations were made from the actual analyses no great change would be made in the

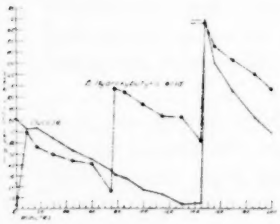

Fig. 1

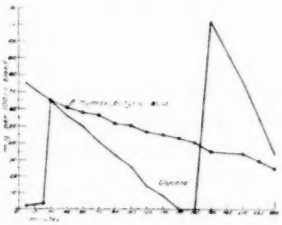

Fig. 2

Fig. 3

Fig. 1. Glucose and ketone bodies calculated as β hydroxybutyric acid in the blood of the dog heart lung.

Dog, weight 15 kgm., 650 mgm. Na β hydroxybutyrate injected at 5 min., the same plus 10 units insulin at 76 min., and the same β hydroxybutyrate plus 5 units of insulin and 2.0 grams glucose at 146 min.

Fig. 2. Glucose and ketone bodies calculated as β hydroxybutyric acid in the blood of the dog heart lung.

Dog, weight 11.3 kgm., 750 mgm. Na β hydroxybutyrate injected at 24 min., 1.0 gram glucose and 5 units insulin at 166 min.

Fig. 3. Glucose and ketone bodies calculated as β hydroxybutyric acid in the blood of the goat heart lung; 750 mgm. Na β hydroxybutyrate injected at 15 min.

figures if the corresponding blood levels had been used for calculation. Blood sugar was measured by the Shaffer-Somogyi (1933) method.

RESULTS. Fourteen experiments have been completed, twelve with the dog and two with the goat. In seven, complete balance studies were made, and in the others the concentration change rates only were noted in relation to the blood sugar level at the time.

Figure 1 shows an example of the disappearance rate of β hydroxybutyric acid from the dog heart lung. Three injections of 650 mgm. each of the sodium salt of this acid[1] were made. The first injection was followed by

[1] A very pure preparation of the racemic salt obtained from the British Drug Houses was employed in all of the experiments reported in this paper.

a decline in concentration at the rate of 0.82 mgm. per cent per minute. With the second injection 10 units of insulin were given. The decline following was at the rate of 0.69 mgm. per cent per minute. With the third injection 2 grams of glucose plus 5 units of insulin were given. The rate of decline thereafter was 1.13 mgm. per cent per minute. The more rapid rate of decline after the first injection than after the second, and in particular the most rapid rate following the third, while the blood sugar was greatly elevated, indicates that the blood sugar level within these limits is not directly correlated with the rate of $\beta$ hydroxybutyrate utilization. In no experiment have we noted an increase in the utilization rate of ketones with lowered blood sugar, with or without the addition of insulin. In another experiment, figure 2, the slope of the blood ketone curve was unchanged while the blood sugar fell to zero and after it was raised by 1 gram of glucose to 112 mgm. per cent. No insulin was given in this experiment during the first three hours.

The goat heart lung was studied in order to extend the observations to another species, particularly because ketone body metabolism is notoriously variable from one species to another. Young goats were used in order to be able to employ the same apparatus used with the dogs. Figure 3 presents the results of one of the two concordant experiments. It will be noted that the rate of disappearance was 0.88 mgm. per cent per minute, a value in the same range as those found for the dog heart at similar blood concentrations. In each of the two cases the circulating blood volume was initially the same, about one liter.

The body weights for the dog in figure 1 and the goat were approximately the same at 15 kgm. In the second experiment with the goat heart the rate of disappearance was somewhat lower. Any comparison of rates of utilization in the two species on the basis of our data is necessarily very rough and we do not wish to insist upon any point other than that the goat heart lung utilizes $\beta$ hydroxybutyric acid.

The important question arises as to what percentage of the total oxidative metabolism of the heart and lungs can be accounted for on the basis of ketone body loss. Before the disappearance of ketone bodies can be considered to be equivalent to their metabolism it must be demonstrated that no acetone is given off in the expired air. Washing this air with bisulfite has led us to a confirmation of Snapper and Grünbaum (1928) in finding absolutely no measurable acetone production from $\beta$ hydroxybutyric acid. Furthermore no acetoacetic acid was formed as is evidenced by the fact that without addition of potassium dichromate to the reaction mixture no acetone complex precipitate occurred which would have been found if acetoacetic acid were present in the blood samples. Consequently $\beta$ hydroxybutyric body disappearance can be considered as equivalent to its metabolism.

The balance studies which have been performed have indicated that the proportion of the total metabolism which is at the expense of β hydroxybutyrate depends upon the concentration of that substance in the blood. A sample experiment with the full data and calculations is presented in table 1. It will be seen that in this experiment 22 per cent of the total oxygen consumption can be accounted for by ketone body loss, assuming that 1.0 mgm. β hydroxybutyric acid requires 0.96 cc. $O_2$ for complete combustion.

TABLE 1

*Typical heart lung experiment on β hydroxybutyric acid utilization*

12-11-37. Dog weight 9.6 kgm.

| TIME | SAMPLE NO. | β HYDROXYBUTYRIC ACID IN BLOOD | REMARKS |
|---|---|---|---|
| | | *mgm. per cent* | |
| 11:40 | 1 | 5.0 | |
| 11:55 | 2 | | 500 mgm. sodium β hydroxybutyrate added |
| 11:57 | | | |
| 12:19 | 3 | | |
| 12:34 | 4 | 22.8 | |
| 12:49 | 5 | 20.5 | |
| 1:04 | 6 | 19.4 | |
| 1:19 | 7 | 17.2 | |
| 1:34 | 8 | 15.5 | |
| 1:52 | 9 | | |
| Blood at end | | 14.5 | 1170 cc. |
| Tissue at end | | 12.2 | 342 grams |

| β hydroxybutyric acid at 11:57: | | β hydroxybutyric acid at end: | |
|---|---|---|---|
| Injected | 412.0 | Removed in samples | 8.1 |
| Present at 11:57 | 75.0 | In blood at end | 170.0 |
| | | In tissues | 41.0 |
| Total | 487.0 | Total | 219.1 |

487 − 219 = 268 mgm. burned or 2.33 mgm./min.

The observed and calculated values for five heart lung experiments are given in table 2. In these experiments the initial blood ketone concentrations following injection ranged from 0.023 to 0.275 per cent. Only in the case of the highest concentration was there evidence that the substance had a deleterious effect upon the heart. In this case the heart dilated and was working at lowered efficiency. With the lower doses the heart evidenced a slight tonic effect, in that it was able to do the same amount of work at a slightly lower diastolic volume. This effect is also noted with glucose and may represent simply the result of an abundance of a readily available fuel.

The largest proportion of ketone metabolism to the total (column 9) was seen at blood ketone concentrations of from 50 to 100 mgm. per cent. The lower proportion found in the one experiment at 275 mgm. per cent we accept with reservations, since we have only one such experiment. If subsequent observation should prove it to be correct it is likely that the toxic effect upon the heart muscle will be found to be in some way responsible.

In order to determine what rôle the lungs might play in the utilization of β hydroxybutyrate lungs were perfused as described above. It was

TABLE 2

*The utilization of β hydroxybutyric acid by the heart and the lungs compared with measured simultaneous oxygen consumption*

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| EXPT. TYPE | DOG WEIGHT | LUNG WEIGHT | INITIAL β HYDROXY-BUTYRATE CONCEN-TRATION | TOTAL TIME | AVERAGE OXYGEN CON-SUMP-TION | β HYDROXY-BUTYRATE DISAP-PEARANCE | MGM. USED BY LUNG PER MINUTE† | 7/6 PER CENT OF TOTAL OXYGEN FOR β-OH‡ |
| | *kgm.* | *gm.** | *per cent* | *minutes* | *cc. per min.* | *mgm. per min.* | | |
| Heart-lung | 17.7 | 142 | 0.275 | 137 | 10.30 | 5.15 | 2.27 | 52 |
| | 8.7 | 69 | 0.095 | 123 | 5.56 | 4.4§ | 1.10 | 82 |
| | 20.5 | 164 | 0.046 | 90 | 7.90 | 5.8 | 1.17 | 76 |
| | 9.6 | 76 | 0.023 | 115 | 8.75 | 2.33 | 1.22 | 22 |
| | 11.4 | 91 | 0.023 | 68 | 12.10 | 2.9 | 1.45 | 25 |
| Pump lung | 16.8 | 135 | 0.066 | 73 | 1.84 | 1.4 | | 80 |
| | 20.5 | 164 | 0.064 | 70 | 1.66 | 1.2 | | 78 |

* Calculated from control data showing lung weight = 0.80 per cent of body weight.

† Calculated from beginning lung weight and mean lung utilization, 0.016 mgm. per gram lung per minute at 0.065 per cent β hydroxybutyric acid concentration.

‡ Represents a rough calculation of the total metabolism accounted for by disappearance of ketone, since 1 mgm. β hydroxybutyric acid uses 0.96 cc. $O_2$ for complete combustion.

§ Mean of two poorly agreeing values.

found, contrary to the statement of Snapper, Grünbaum and Neuberg (1926) who describe the results of a single experiment on the perfusion of a cat lung with calves' blood and found a slight, but in their opinion negligible, disappearance of β hydroxybutyric acid, that in our hands a considerable proportion of the lung metabolism is accounted for by ketone destruction. We found that approximately 80 per cent of the total is at the expense of this substance when the blood β hydroxybutyrate concentration is raised to 65 mgm. per cent. It may be that Snapper, Grünbaum and Neuberg were misled by the smallness of the total metab-

olism of lung tissue, or they may not have aerated the system properly. It is not impossible moreover that the use of heterologous blood influenced their result. In any case we are forced to admit that a considerable proportion of the total β hydroxybutyrate lost from the heart lung was probably burned in the lungs themselves. Calculating that 80 per cent of the total metabolism of the lungs is at the expense of β hydroxybutyrate, which is not unreasonable since we found this figure at 65 mgm. per cent blood concentration, we find as shown in column 8 in table 2, that from 1.10 to 2.27 mgm. per minute might conceivably have been burned by the lungs and blood. These quantities are, however, only from 20 to 50 per cent of the total used by the heart lung preparation. We are obliged to conclude, therefore, that the heart muscle itself must have metabolized from 50 to 80 per cent of the ketones which disappeared.

Discussion. The observation that heart muscle can utilize β hydroxybutyric acid, presumably by complete oxidation, since no acetone is found to be evolved in the system, is not surprising. The fundamental metabolism of striated and cardiac muscle has not been shown, to our knowledge, to be in any way qualitatively different in the two tissues. The observations of Snapper and Grünbaum (1928) on striated muscle are clear-cut in demonstrating ketone body utilization. Cruickshank (1936) has reviewed the literature on the question of fat burning by muscle, particularly cardiac. He calls attention to the low R.Q. of muscle metabolism in the absence of adequate carbohydrate. He does not mention in this connection the crucial experiments by Bayliss, Müller and Starling (1928) on the heart, who found respiratory quotients at or below 0.85 before additions of glucose or insulin. The heart does not metabolize protein or amino acids according to Cruickshank and McClure (1936), therefore it is hard to escape the conclusion that fat is being burned. Visscher and Mulder (1930) have attacked this question from another angle. They made carbohydrate balance studies on the heart lung and measured the total oxygen consumption. They found that as much as 80 per cent of the total metabolism is at the expense of something other than the carbohydrate found to disappear. Since they found no evidence of increase in non-protein nitrogen they concluded that fat must have been burned.

No work was cited by Cruickshank on the direct measurement of fat disappearance from heart muscle. In previous experiments Visscher (1938) has found in a study of over sixty hearts that the mean total fat in ventricular muscle in unworked hearts was 3.71 per cent. In twenty-five hearts worked in the heart lung from two to eight hours the value was 3.15 per cent. The difference between the means was 0.53 per cent. The probable error of the difference between the means was 0.12 per cent. Since the difference found was 4.4 times this value the probability is very

great that the difference is real. Although alternative interpretations have been discussed the most reasonable interpretation is that the heart muscle had burned this proportion of its fat. It is interesting to note that 0.53 gram of fat would require about 1 liter of oxygen for complete combustion. The hearts were a little less than 100 grams in weight and worked an average of 3 hours. They used some 600 cc. of oxygen per hour, therefore the fat disappearance would account for about two-thirds of the total metabolism. These experiments were conducted without additions of glucose and the blood sugar levels were consequently abnormally low. It is likely that less fat would have been burned if more carbohydrate had been available. It may be noted that in the ten comparable experiments of Visscher and Mulder (1930) the mean value for the proportion of the total metabolism which was non-carbohydrate was found to be 63 per cent. This rough agreement between calculations from the fat loss and the non-carbohydrate metabolism appears to be of some significance.

The preponderance of evidence is in favor of direct oxidation of fat by heart muscle in the absence of an abundance of carbohydrate. The present paper demonstrates, we believe, that the intermediate substance, $\beta$ hydroxybutyric acid, is also capable of being burned by the isolated heart in the heart lung preparation.

## CONCLUSIONS

1. Added $\beta$ hydroxybutyric acid disappears from the blood of the heart lung preparation of the dog and the goat.

2. The rate of disappearance was not found to be related to the blood sugar level at the time.

3. Balance studies show that the rate of utilization rises with increased concentration in the blood up to 100 mgm. per cent.

4. As much as 80 per cent of the lung metabolism may be at the expense of $\beta$ hydroxybutyric acid metabolism.

5. No acetone in detectable quantities is given off in the expired air in the heart lung preparation after additions of $\beta$ hydroxybutyric acid, and no acetoacetic acid is found in the blood.

6. As large a proportion as 82 per cent of the total oxygen consumption of the heart lung may be due to $\beta$ hydroxybutyrate burning when its concentration is approximately 100 mgm. per cent in the blood.

7. Other evidence is cited to show that the heart muscle is capable of direct oxidation of fat to the extent of at least two-thirds of its total metabolism.

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